Analysis of Novel Haemotrophic *Mycoplasma* Isolates with Main Focus on Significance of Haemotrophic *Mycoplasma* Infections in Horses

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LIST OF ABBREVIATIONS

A.	*Anaplasma*
AAEP	American Association of Equine Practitioners
acc. no.	accession number
Ae.	*Aedes*
AG	'Aktiengesellschaft'
approx. / ca.	approximately
B.	*Bartonella*
Ba.	*Babesia*
Bact. Load	bacterial load
bp	base pair
BSA	bovine serum albumin
°C	degree Celsius
CARD-FISH	Catalysed reporter deposition fluorescence *in situ* hybridisation
CH	Switzerland
Ch.	Chapter
*C*M	*Candidatus* Mycoplasma
CO_2	carbon dioxide
conc.	concentration
CP	crossing point
Cy5	cyanine
D.	*Dermacentor*
DAPI	4′,6-diamidin-2-phenylindol
dL	deci litre
DMF	dimethylformamide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
E.	*Eperythrozoon*
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
e.g.	*exempli gratia* (for example)
ELISA	Enzyme-linked immunosorbent assay
EIA	equine infectious anaemia
et al.	*et alii* (and others)
EtBr	ethidium bromide
EtOH	ethanol
Fig.	Figure
FITC	fluorescein isothiocyanate
fL	femtolitre
g	gram / acceleration of gravity
G/L	10^9/L
GA	glutaraldehyde
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GER	Germany
h	hour(s)
H.	*Haemobartonella*
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HCl	hydrochloric acid
HM	haemotrophic mycoplasmas
HRP	horseradish peroxidase
Ht	haematocrit
I.	*Ixodes*
IAC	isoamyl alcohol:chloroform (1:24)
IgG / IgM	immunoglobulin G/ M
IPTG	isopropyl β-D-1-thiogalacto-pyranoside
IUPAC	International Union of Pure and Applied Chemistry
IVB	Institute of Veterinary Bacteriology
K_2PO_4	potassium phosphate
kb	kilo base pair
L	litre
M	molar
M.	*Mycoplasma*
mA	milliampere
MCH	mean cell haemoglobin
MCHC	MCH concentration
MCV	mean cell volume
Me.	*Melophagus*
mg	milligram

µg	micro gram
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
min	minute(s)
mL	millilitre
µL	microlitre
mM	millimolar
µm	micrometer
mmol	millimol
MSG1	*M. suis* GAPDH-like protein 1
n/a	not available
NA	gram reaction is not applicable
NaAc	sodium acetate
NaCl	sodium chloride
NAD	Nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NAS	nucleic acid sequence
NC	not cultivable / nutritional condition
n.d.	not determined
neg	negative
ng	nanogram
nm	nanometre
no. / n	number
n.s.	not significant
O	other (morphology)
P	probability
P.	*Polyplax*
PBS	phosphate buffered saline
PBS-GB	PBS, supplemented with glucose and BSA
PBS-T	PBS, supplemented with Triton X-100
PCR	polymerase chain reaction
pers. comm.	Personal communication
PFA	paraformaldehyde
pg	picogram
pmol	picomol
pos	positive
p(p).	page(s)
1st Qu	first quartile
3rd Qu	third quartile
R.	*Rhipicephalus*
RBC(s)	red blood cell(s)
RBCC	red blood cell count
RNA	ribonucleic acid
rRNA	ribosomal RNA
rnpB	gene of the RNase P RNA
rpm	rounds per minute
rpoB	gene of the β subunit of the bacterial RNA polymerase
r_S	Spearman rank correlation coefficient
RT	room temperature
S	symbiotic (habitat)
S.	*Stomoxys*
SDS	sodium dodecyl sulphate
s	second(s)
SEM	scanning electron microscopy
sp.	species
T.	*Theileria*
T/L	10^{12}/L
Tab.	Table
TAE	tris-acetate-EDTA
T_M	melting temperature
TM	trademark
Tris	tris(hydroxymethyl) aminomethane
tRNA	transfer RNA
TU(M)	'Technische Universität (München)'
U	unit(s)
USA	united states of America
UV	ultraviolet
WBC(s)	white blood cell(s)
WBCC	white blood cell count
X-Gal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside

I. SUMMARY

I. SUMMARY

I. 1 Summary

Haemotrophic mycoplasmas (HM), small, cell wall-less, uncultivable bacteria, reside on the surface of mammalian red blood cells (RBCs). They were first described in the early 20th century as *Eperythrozoon* and *Haemobartonella* species within the group of *Rickettsia*. In the late 1990s, they were reclassified as *Mycoplasma* species within the group of *Mollicutes*. In most animal species, a HM infection is accompanied by haemolytic anaemia, and clinical signs may include decreased exercise tolerance, lack of appetite, poor weight gain, and infertility.

Although HM infections have already been studied in several domesticated and wild animals, almost nothing is known about possible infections in horses. First indications of the existence of horse-specific HM parasites and their infection of horses in the Luneburg Heath (Northern Germany) were reported by owners presenting horses showing the aforementioned clinical signs. Blood samples of these animals were analysed for HM using microscopy, PCR, and 16S rRNA sequencing, and the first molecular proof, that HM infections do occur in horses, was shown.

The clinical significance of HM infections in horses was evaluated. Blood samples from horses of one breeding farm were analysed haematologically and screened using PCR and microscopy. For a more sensitive and unambiguous diagnostics, a specific SYBR green I real-time PCR assay was developed. A high prevalence of 33.2 % and a mean blood load of 1.67×10^7 cells/mL blood were observed. Infected horses older than one year showed no significant blood parameter changes, while those younger than one year showed a significant reduction in red blood cell count, haematocrit, and haemoglobin concentration, indicating a haemolytic anaemia. In horses, HM infections frequently displayed a subclinical course of disease, with relatively low concentrations of bacteria in blood. A severe course of disease with pronounced clinical signs and anaemia may only develop in young, stressed, or immunocompromised animals.

Generally, the current classification of HM in the *Mycoplasma*-cluster in the *Mollicutes* group was confirmed, and within HM's group, two subclusters were found ('haemofelis-group' and 'haemominutum-group'). In Northern Germany, two HM isolates were found in cattle: *M. wenyonii* and '*C*M haemobovis'. The novel equine HM isolate belongs to the 'haemofelis-group' and shows 97-98 % 16S rRNA identity to '*C*M haemobovis'.

I. 2 Zusammenfassung

Hämotrophe Mykoplasmen (HM) sind kleine, zellwandlose, unkultivierbare Bakterien auf der Erythrozyten-Oberfläche von Säugetieren. Sie wurden Anfang des 20. Jahrhunderts erstmals beschrieben und als *Eperythrozoon* oder *Haemobartonella* innerhalb *Rickettsia* klassifiziert. In den späten 1990iger wurden sie als Mykoplasmen (*Mollicutes*) reklassifiziert. In den meisten Tierarten ist eine HM-Infektion durch hämolytische Anämie, Leistungsabfall, Appetitlosigkeit, Gewichtsverlust und Infertilität gekennzeichnet.
Obwohl HM-Infektionen in vielen Haus-, Nutz- und Wildtieren beschrieben wurden, ist bisher fast nichts über eine mögliche equine Infektion bekannt. In der Lüneburger Heide (Norddeutschland) wurde wiederholt von anämischen Pferden mit oben genannter Symptomatik berichtet. Blutproben dieser Tiere wurden auf das Vorkommen von HM mithilfe von Mikroskopie, PCR und 16S rRNA-Sequenzierung untersucht. Dabei konnte der Erreger erstmals zweifelsfrei molekularbiologisch im Blut von Pferden nachgewiesen werden.
Um die klinische Relevanz einer HM-Infektion beim Pferd zu untersuchen, wurden sowohl große Blutbilder angefertigt, als auch Mikroskopie und PCR durchgeführt. Zur sensitiveren und zweifelsfreien Diagnostik wurde ein spezifischer SYBR green I real-time PCR Assay entwickelt. Dabei wurde eine relativ hohe Prävalenz (33.2 %) mit einer durchschnittlichen Bakterienanzahl von 1.67×10^7 Zellen/mL Blut gefunden. Während bei infizierten Pferden älter als ein Jahr keine signifikanten Veränderungen im Blutbild beobachtet wurden, zeigten infizierte Pferde unter einem Jahr signifikante Reduzierungen der Erythrozytenzahl, des Hämatokrit und der Hämoglobin-Konzentration. Dies deutet auf eine hämolytische Anämie hin. Im Grossen und Ganzen scheint diese Infektion in Pferden eher subklinisch mit einer nur geringen Anzahl von Bakterien im Blut zu verlaufen, während die akute Verlaufsform mit ausgeprägter Symptomatik und Anämie nur in jungen, gestressten oder immunkompromitierten Tieren vorzukommen scheint.
Im Allgemeinen wurde die Eingruppierung der HM in die Gruppe der *Mollicutes* innerhalb des Mykoplasmen-Clusters bestätigt. Es wurden zwei Subcluster innerhalb der HM gefunden (‘haemofelis-’ und ‘haemominutum-Gruppe’). In Norddeutschland wurden in einer anämischen Rinderherde zwei verschiedene HM Isolate gefunden: *M. wenyonii* und ‘*C*M haemobovis’. Das neue equine HM Isolat gehört zur ‘haemofelis-Gruppe’ und zeigt auf Ebene der 16S rRNA eine 97-98 %ige Identität zu ‘*C*M haemobovis’.

II. INTRODUCTION

II. INTRODUCTION

II.1 The genus Mycoplasma

Mycoplasmas are the smallest self-replicating bacteria known thus far (0.3-1.0 µm), near the theoretical minimum cell size limit of 0.15 µm (195, 256, 259, 261). Mycoplasmas' genomes range in size from 580 kb (*M. genitalium*, 89) to 1359 kb (*M. penetrans*, 272) and code for up to only 700 proteins (258). Mycoplasmas are also characterised by a low GC % content (23-41 %, 195) and unusual codon usage (261, 262). Due to their small size, they pass through common bacterial filters and were therefore not detected until 1898 ('microbe de la péripneumonie', 234). In 1929, NOWAK introduced the designation '*Mycoplasma*' (237). Although gliding motility was reported for some species (e.g. *M. pneumoniae*, *M. gallisepticum*, and *M. pulmonis*) (195), most mycoplasmas are non-motile. In mycoplasmas, a cell wall is missing, leading to various cell and colony shapes (fried-egg shaped colonies, pear-shaped or flask-shaped cells, terminal tip structures, and filaments) (256, 261). Cell shapes are governed by cytoskeleton-like structures (261, 262), but may be influenced by culture and procession conditions (195). Until the first genomic analysis in the 1960s, the scientific consensus was that mycoplasmas may represent L-forms of bacteria (bacteria which have lost their cell wall) (218). Genomic analyses, however, revealed no relationship to known cell-walled bacteria forming L-forms (262).

Their stringent nutritional requirements render them difficult to cultivate, so many species have not yet been cultivated (8, 261), hampering research and diagnostics. Several explanations for this phenomenon were offered based on results from genomics experiments. Both, *M. genitalium* and *M. pneumoniae*, lack genes for amino acid synthesis (89, 124, 260), requiring very complex media including various growth supplements (256, 262). Due to their small genomes, mycoplasmas do not have an extensive metabolism; they only have enzymes for energy production. Furthermore, mycoplasmas have a limited respiratory system, as they lack quinones, cytochromes, and parts of the citric acid cycle (250, 256, 258, 260, 261). Despite their small genomes and stringent environmental requirements, mycoplasmas are widely distributed and common pathogens due to their high level of adaptation to certain tissues in various hosts (plants, insects, animals, and humans) (8, 255, 256, 337). They rely on an obligate parasitic lifestyle and occur as extra- and intracellular parasites (261, 262).

II.2 Haemotrophic mycoplasmas

II.2.1 Overview

Haemotrophic mycoplasmas (HM) are extraordinary members of the large group of mycoplasmas due to their obligate intra- or extracellular parasitism of mammalian red blood cells (RBCs) (106, 136, 172, 210). Deformity and destruction of RBCs caused by attached HMs lead to mild to severe haemolytic anaemia (136, 210). Since HMs require special growth conditions, *in vitro* cultivation has not yet been successful, although an *in vitro* maintenance system for *M. suis* was proposed (235). Thus, researchers still rely on ethically questionable experimental infections of splenectomised or otherwise immunocompromised animals to analyse HM's physiology, pathogenesis, and immunology (34).

First representatives of HMs were discovered in 1928 in Germany, when SCHILLING observed roundish RBC parasites in mice and named them *Eperythrozoon coccoides* (275). In 1939, TYZZER & WEINMAN detected *Haemobartonella muris* in mice (342). Since then, many novel *Eperythrozoon* and *Haemobartonella* species in several animal hosts were found all over the world (210). In the late 1990s, *Eperythrozoon* and *Haemobartonella* species were reclassified as *Mycoplasma* species within the *Mollicutes* class (e.g. 219, 220, 265; Ch. II.2.7., p. 22). An increasing number of case reports within the past several years demonstrate how HM infections are not restricted to farming and companion animals (e.g. pigs, cattle, dogs, and cats) anymore; HM isolates were also found in wild animals such as lions (108) and capybara (350). Furthermore, novel species in cats (360), cattle (314), sea lion (351), and deer (355) were detected.

HM infections merit further study due to their economic and scientific impact. In livestock husbandries, HM infections can lead to important economic losses: decreased weight gain in pigs, decreased wool production in sheep, and decreased fertility (53, 136, 210, 212). Undetected latent HM infections in laboratory animals may cause interferences and bias of experiments (e.g. dog (164), mice (25, 99, 245, 266, 275), rats (75), and primates (64, 221)).

II.2.2 Pathogenesis and clinical signs

HMs induce a disease known by several names ('eperythrozoonosis' (122, 263, 292), 'haemobartonellosis' (35, 192), 'infectious anaemia' (116, 136), and 'haemoplasmosis' (315)), but each describes more or less the same clinical manifestation. The incubation period varies from days to several weeks (312). The most prominent clinical sign in infected animals is haemolytic anaemia; HMs attach to RBCs, which are then deformed and destroyed (136, 138, 210). Further possible clinical signs are summarised in Table II-1 (pp. 9-10).

HM infection severity can vary from mild to life-threatening. Severity could possibly be linked to the number of bacteria in blood (182, 325), although this point remains a subject of contention (322, 361). Furthermore, a subclinical course of disease and chronic infection states have been reported, and infections may persist chronically for years until an outbreak is induced (85, 86, 136, 194, 210, 289, 292). Chronic infections are typically observed in non-splenectomised, immunocompetent animals (210). Splenectomy or immunocompromisation of latent infected animals can induce severe disease (35, 99). Chronically infected animals may represent HM reservoirs and could possibly spread the infection to other animals. Mainly immunocompromised, stressed, pregnant, and juvenile or old animals are affected (99, 105, 136, 199, 210, 311, 336). Severity of disease can also be affected by interference with concurrent infections, e.g. anaplasmosis (*Anaplasma marginale, A. ovis*) (112, 139, 140), piroplasmosis (*Theileria* sp., *Babesia* sp.) (65, 139, 245), virus infections (29, 46, 57, 86, 95, 103, 105), and co-infections of multiple HM species (154, 202, 323, 361). Additionally, interactions of mycoplasmas with the host immune system should be considered (261).

HMs adhere to RBCs in a multifactorial process involving many accessory proteins (261), and they can attach to RBCs via fibrillary structures (64, 247, 376). In *M. gallisepticum, M. imitans*, *M. genitalium, M. pneumoniae*, and *M. alvi*, a terminal tip structure was detected (30, 39, 124, 256, 261, 285), but this structure is absent in *M. hominis, M. fermentans, M. pulmonis*, and *M. suis* (40, 106, 261, 376). Attachment of *M. penetrans* induces a cytoskeletal rearrangement in the host cell (97). Adhesion proteins in *M. pneumoniae* and *M. genitalium* have also been observed and characterised (89, 124, 125, 261). RAZIN stated that there may be a "conserved family of mycoplasmal cytadhesins" (261). It appears that metabolic exchange between HMs and RBCs occurs, as "close contact seems to be crucial for the life cycle of this bacterium" (136). In 2007, HOELZLE *et al.* proposed MSG1 (*M. suis* GAPDH-like protein 1), a protein highly

similar to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as a putative adhesion protein of *M. suis* (135). GAPDH is an enzyme involved in glycolysis, and hypoglycaemia is well connected with *M. suis* metabolism (119, 235, 294). Massive metabolic disturbances were observed in pigs as well as in sheep. In these cases, increased lactate and pyruvate concentrations alongside decreased bicarbonate concentrations in blood lead to severe blood acidosis (119, 120, 148). Hypoglycaemia was also observed in llamas showing peak parasitaemia (6, 199). Parasitaemia can range in infection severity, from barely detectable to more than 85 % of RBCs affected (163). Each RBC can carry up to twenty or more HM cells. Attached HMs lead to deformation of RBC membrane, and affected RBCs display pits, trenches, invaginations and indentations (163, 219, 252, 265, 316, 376), which induce a type-II autoimmune reaction and lead to a Coombs' positive immune mediated haemolytic anaemia (35, 136, 192). In pigs (*M. suis*; 138, 156, 276), mice (*M. coccoides*; 150), cats (*M. haemofelis*; 328, 380), and dogs (*M. haemocanis*; 17), high titres of IgM cold agglutinins were found. IgM cold agglutinins are either directed as auto-antibodies against RBC surface antigens or against bacteria attached to the RBC surface. In the latter case, destruction of RBCs would be a side effect. Activity of cold agglutinins may directly result in clinical signs such as acrocyanosis and pallor (17, 35, 48, 136, 156, 276, 377, 380). Additionally, HMs could directly damage RBCs. Our institute has performed initial studies which support the existence of this pathway; warm auto-reactive IgG antibodies were found in *M. suis*-infected pigs, which are specifically directed against porcine actin, leading to eryptosis and resulting in severe haemolytic anaemia (78).

Since HM are not cultivable, pathogenicity studies of HM are complicated and the exact mechanism of adhesion, attachment, invasion, and destruction of RBCs is an active area of research.

Table II-1 Clinical signs of HM infections

Clinical Sign	HM species	References
abortion	*M. suis*	210
acrocyanosis	*M. suis*	118
anaemia	'*C*M haemolamae', *M. ovis*, *M. suis*, *M. haemofelis*, *M. wenyonii*, '*C*M turicensis'	6, 36, 53, 122, 136, 137, 210, 263, 312, 360
anorexia	'*C*M haemolamae', *M. suis*, *M. haemofelis*, *M. wenyonii*	6, 136, 210, 212, 263
bacteraemia	*M. suis*, *M. haemofelis*	312, 377
bile-coloured faeces	*M. suis*	118
cardiac murmurs	*M. haemofelis*	312
collapse	'*C*M haemolamae'	210
cyanosis	*M. suis*	118
decreased exercise tolerance	*M. ovis*	36, 53, 210
decreased milk production	*M. suis*, *M. wenyonii*	136, 210, 293
decreased wool production	*M. ovis*	36, 53
dehydration	*M. haemofelis*	312
depression	'*C*M haemolamae', *M. suis*, *M. haemofelis*	6, 210, 312
diarrhoea	'*C*M haemolamae'	6, 263
dyspnoea	'*C*M haemolamae', *M. suis*	6, 118
oedema	*M. wenyonii*	212
enteritis	*M. suis*	118
fever	*M. suis*, *M. haemofelis*, *M. wenyonii*	118, 136, 210, 212, 312
hepatomegaly	*M. suis*	169
hyperkalaemia	'*C*M haemolamae'	6
hypoglycaemia	*M. suis*, *M. ovis*, '*C*M haemolamae'	6, 119, 184, 199, 210, 294, 306
hypoproteinaemia	'*C*M haemolamae'	263
hypothermia	*M. haemofelis*	312
icterus	*M. suis*, *M. ovis*, *M. haemofelis*	122, 136, 169, 210, 312
ill-thrift	*M. suis*, *M. ovis*	36, 53, 136, 210

Table II-1 continued

Clinical Sign	HM species	Reference
immune suppression	*M. suis*	136, 377
inappetence	*M. suis*, '*C*M haemolamae', *M. haemofelis*	6, 118, 136, 312
infertility	*M. suis*, *M. wenyonii*	136, 210, 212
lethargy	*M. haemofelis*, '*C*M haemolamae', *M. wenyonii*	210, 212
lymphadenopathy	*M. wenyonii*	210, 293
metabolic acidosis	'*C*M haemolamae'	6
pale mucous membranes	*M. ovis*, *M. haemofelis*	210, 312
parasitaemia of RBCs	*M. ovis*, '*C*M haemolamae', *M. haemofelis*	6, 136, 137, 210, 312
petechial haemorrhages	'*C*M haemolamae'	6
poor body condition	'*C*M haemolamae'	263
poor maternal behaviour	*M. suis*	136, 210
poor weight gain	*M. suis*, *M. ovis*	36, 53, 136, 210
pyrexia	*M. suis*	210
rough haircoat	*M. wenyonii*	210, 293
skin pallor	*M. suis*	118, 136
splenomegaly	*M. suis*, *M. haemofelis*	169, 312
tachycardia	*M. wenyonii*, *M. haemofelis*	212, 312
tachypnoea	*M. wenyonii*, '*C*M haemolamae', *M. haemofelis*	6, 212, 312
unthriftiness	'*C*M haemolamae', *M. suis*	6, 136
weakness	'*C*M haemolamae', *M. ovis*, *M. haemofelis*	6, 137, 312
weight loss	*M. ovis*, '*C*M haemolamae', *M. wenyonii*, *M. haemofelis*	137, 210, 263, 312

II.2.3 HM infection of different animal species

HM infections occur in a variety of animal hosts, as listed in Table II-2.

Table II-2 Haemotrophic *Mycoplasma* species and their hosts in chronological order of their first isolation

Species	Synonym	Host animal	Reference
M. coccoides	*E. coccoides*	Rodents	224, 275
M. haemocanis	*H. canis, B. canis*	Dog	167, 208
M. wenyonii	*E. wenyonii*	Cattle	2, 219
	H. bovis, B. bovis[a,b]	Cattle	66
M. ovis	*E. ovis*	Sheep, Goat	223, 226
M. haemomuris	*H. muris*	Rodents	220, 222, 342
M. suis	*E. suis*	Pig, wild boar	1, 69, 128, 169, 220, 222, 296
	E. parvum[a,b]	Pig	297, 299
M. haemofelis	*H. felis, E. felis*	Cat, wild felids	45, 82, 220, 222, 365
	E. teganodes[a,b]	Cattle	143
	E. tuomii[a,b,c]	Cattle	339, 343
	H. procyoni[a,b]	Racoon	90
	E. maribori[a,b]	Fruit bat	76
'*C*M haemolamae'		Alpaca, Llama	199, 208, 263
'*C*M haemominutum'		Cat, wild felids	86, 365
'*C*M haemobovis'		Cattle	139, 314
'*C*M haemodidelphis'		Opossum	207, 208
'*C*M kahanei'		Squirrel monkey	221
'*C*M haematoparvum'		Dog	165, 308, 309
'*C*M turicensis'		Cat, wild felids	360, 365
'*C*M haemocervae'		Sika deer	355
'*C*M erythrocervae'		Sika deer	355
'*C*M haemozalophi'		Sea lion	351

[a] = not affiliated in the 'Approved Lists of Bacterial Names' (http://www.bacterio.cict.fr/; 287); [b] = no DNA available; [c] = observed on the surface of thrombocytes

II.2.3.1 Rodents

The first HM species to be discovered was *M. coccoides* (formerly *E. coccoides*; 224), and it became the type species of the former genus *Eperythrozoon* (275). *M. coccoides* induces an intense parasitaemia resulting in haemolytic anaemia in rodents (48, 98, 332, 333).

A second HM species in rodents was described in 1939 by TYZZER & WEINMAN (342) and characterised in detail by RIKIHISA *et al.* in 1997 (265). *M. haemomuris* (formerly *H. muris*) shows 89 % and 85 % 16S rRNA identity to *M. haemofelis* and '*C*M haemominutum', respectively, so a reclassification as a *Mycoplasma* species was proposed (265). *M. haemomuris* exhibits a higher pathogenic potential than *M. coccoides* and may

induce fatal disease or chronic carrier states in albino rats, albino mice, wild mice, and hamsters.

In Brazil, an HM isolate was found in capybaras (*Hydrochaeris hydrochaeris*) that is closest related to *M. coccoides* (92 % 16S rRNA sequence identity) and '*C*M turicensis' (91 %). Detection of a new species was postulated, but no name was given (350).

II.2.3.2 Dog

In 1928, an anaemia-inducing infective agent similar to *Bartonella* was found in dogs (167). Since then, cases of canine 'haemobartonellosis' have been reported worldwide (18, 103, 179), although infections do not play a significant role in healthy dogs (17, 32, 164). Recently, *H. canis* was reclassified as *M. haemocanis* (208). Despite *M. haemocanis*' 99 % 16S rRNA sequence identity to *M. haemofelis* (208), sequence information of *rnpB* gene shows less similarity (26, 243, 324). Thus, one could justify that both HM isolates represent distinct species (26, 243), as it was suggested by cross-infection studies (84). LUMB'S cross-infection studies showed that cats may act as asymptomatic carriers of *M. haemocanis*: He transmitted blood of dogs diseased with 'haemobartonellosis' into cats. The cats remained asymptomatic, but susceptible dogs became anaemic after blood transfusion of these cats (189).

In 2004, a second canine HM species, '*C*M haematoparvum' (165, 308, 309), was discovered. This novel species is most closely related to '*C*M haemominutum' (94 % 16S rRNA identity) and exhibits only slight similarity to *M. haemocanis* (165, 308, 309). Investigations concerning differences in pathogenicity are currently underway.

II.2.3.3 Pig

"A rickettsia-like or anaplasmosis-like disease in swine" was first described by KINSLEY (169) and DOYLE in 1932 (69). Since this agent displayed striking morphological similarities with then-known RBC parasites (*E. wenyonii*, *E. ovis*), it was named *E. suis* in 1950 (296-298), only to later be reclassified as *M. suis* (1, 220, 222, 265). Currently, scientific consensus pins *M. suis* as the cause for 'porcine eperythrozoonosis' (122, 298), also known as 'infectious anaemia of the pig' (136). The clinical manifestation varies from subclinical to mild anaemia to severe life-threatening course of disease (122, 136, 292), causing high economic losses in pig husbandries (136).

A second, smaller pig-infecting species besides the well-researched *M. suis* (174) is known to infect pigs, *E. parvum*, but this organism appears to have no clinical relevance (13, 270, 281, 297, 299, 345).

II.2.3.4 Cattle

In cattle, five HM species are known: *M. wenyonii* (2, 219), *E. teganodes* (9, 112, 143), *E. tuomii* (339, 343), *H. bovis* (33, 66), and '*C*M haemobovis' (129, 139, 314).

ADLER & ELLENBOGEN discovered the first HM species in cattle, *E. wenyonii*, in 1934 (2). In 1963, KREIER & RISTIC showed that *M. wenyonii* cannot be transmitted to other species (172), although sheep can act as paratenic host (144). In 1997, *E. wenyonii* was one of the first species to be reclassified as a *Mycoplasma* species (219).

'*C*M haemobovis' was first detected in Switzerland (139) and Japan (314) in the beginning of the 21st century. Based on 16S rRNA analysis, a closer relationship to *M. haemofelis* (95 %) than to *M. wenyonii* (84 %) was found (139, 202). TAGAWA *et al.* used analysis of clinical signs and haematological measurements to determine that this novel species is more pathogenic than *M. wenyonii* (315). Also, concurrent *M. wenyonii* and '*C*M haemobovis' infections have been reported (315).

In the pre-sequencing era, three more HM species of cattle were described by means of morphological and infectious criteria: *E. teganodes* (9, 112, 143), *E. tuomii* (339, 343), and *H. bovis* (33, 66). *E. teganodes* and *H. bovis* were found to be more free in the plasma in comparison to *E. wenyonii* (33, 143). *E. tuomii*, on the other hand, attaches to the surface of thrombocytes (339, 343). These three species were clearly distinct form *E. wenyonii* as shown by cross-immunity tests and immunogenic and morphological differences (144, 340). HOYTE found no difference in pathogenicity between *E. wenyonii* and *E. teganodes* (143). Today, these three species have no clinical impact (33, 162). Due to missing DNA of *E. teganodes*, *E. tuomii*, and *H. bovis*, their classification cannot be confirmed by sequencing of phylogenetic marker genes. Thus, they were not included in the 'Approved List of Bacterial Names' in contrast to *E. wenyonii* (129, 287). UILENBERG proposed that '*C*M haemobovis' may demonstrate one of these earlier described HM species (348). However, this statement cannot be conclusively proven without DNA.

II.2.3.5 Cat

Feline HM species are among the best-researched HM species. There are three known feline HM species: *M. haemofelis* (82), '*C*M haemominutum' (85, 86), and '*C*M turicensis' (360), which are distributed worldwide and can infect both domestic and wild felids (108, 344, 354, 365). The species can be clearly distinguished by 16S rRNA sequences (85, 205, 265, 320) and differ in pathogenicity (85, 154, 328, 359, 361).

M. haemofelis may cause severe life-threatening haemolytic anaemia ('feline infectious anaemia') (21, 116). It was first described by CLARK in 1942 as *E. felis* (45) and FLINT & MOSS in 1953 as *H. felis* (82), when they found cats contracted with infectious anaemia. Cross-infection studies revealed no infectivity for rats, mice, swine, cattle, sheep, and dogs (295).

FOLEY *et al.* found an HM-like organism in a cat co-infected with feline leukaemia virus. This organism was much smaller than the already known *M. haemofelis* (*H. felis*) and was initially described as '*H. felis* small form' ('*H. felis* California strain'; 0.3 µm) to distinguish it from the then-known '*H. felis* large form' ('*H. felis* Ohio strain'; ca. 0.6 µm) (85, 265). RIKIHISA *et al.* proposed that these two isolates demonstrate two distinct species, as they only share 85 % 16S rRNA sequence identity and also significantly differ in pathogenicity (86, 154, 265). The name '*C*M haemominutum' was established for the '*H. felis* small form' (86) and *M. haemofelis* for the '*H. felis* large form' (220, 222). Infections with '*C*M haemominutum' result in minor clinical signs or may be even asymptomatic. Blood parameters are not or only slightly changed, but stay within the reference range (85, 86, 359).

Recently, a third feline HM species was described by WILLI *et al.* (360). The species received the name '*C*M turicensis', because it was first isolated in Zurich ('*Turicum*') (360). Since then, '*C*M turicensis' infections have been reported in domestic and wild felids worldwide (91, 361, 362, 364, 365). Their pathogenic potential strongly depends on additional factors like co-infection with other HM species or viruses, immunosuppression, and host susceptibility. '*C*M turicensis' seems to be of lower pathogenicity than *M. haemofelis* but higher compared to '*C*M haemominutum' (311, 360, 364).

Concurrent infections with two or three feline HM species have been reported in domestic (91, 154, 325, 361, 362) and wild felids (108, 365).

II.2.3.6 Sheep and goat

E. ovis was first described in 1943 (153) and reclassified in 2004 as *M. ovis* (223). It displayed a high 16S rRNA sequence identity (95%) with *M. wenyonii* (223), and was shown to have a broader host range infecting also goats, blesbok, eland, and deer (51, 172, 223, 227, 304). However, *M. ovis* cannot induce disease in dogs, guinea pigs, rabbits, and cattle, although the pathogen can persist in bovine blood for a brief time (144, 226).

Recently, HORNOK *et al.* found an ovine HM isolate showing a 17 bp deletion in the 16S rRNA sequence compared to the classical *M. ovis* isolate. If this difference is sufficient to designate a new species, then it will be called '*C*M haemovis' (140).

II.2.3.7 Llama and alpacas

In 1990, first indications of an HM species exclusive to llamas and alpacas were reported in the USA (199, 263). Based on the observation of coccoid and ring-shaped basophilic organisms on the surface of RBCs and free in the plasma, REAGAN *et al.* concluded that, due to morphological similarities to *M. wenyonii*, this may represent a novel *Eperythrozoon* species (263). 16S rRNA sequencing indeed revealed the discovery of a novel species, and '*C*M haemolamae' designation was introduced (199, 208). Clinical manifestations vary from asymptomatic to severe disease with fatal outcome. REAGAN *et al.* reported in their study that mainly young animals were affected (263).

II.2.3.8 Human and primate HM

Also in monkeys (64, 221, 246, 247) and humans, first indications of HM infections were found. In humans, 'haemoplasmosis' occurs mostly in conjunction with concurrent diseases, like systemic lupus erythematosus (158), AIDS (67, 72), mononucleosis (254), and, in one case, a multiple sclerosis patient co-infected with *Bartonella* sp. (313). *M. suis* was found amongst farm workers in China (375) and humans in Inner Mongolia (373). Thus, a zoonotic potential of HM should be kept in mind. TASKER *et al.* aimed to design a real-time PCR assay for detection of all known HM species (329). They tested blood of about 1000 patients for presence of HM and found only one sample to be positive. However, they failed to detect HM in DNA isolated from blood smears obtained from previous studies in which presence of HM in human blood was proposed using cytological analysis (44, 102, 373). Thus, reports of human 'haemoplasmosis' only based on evaluation of stained blood smears without molecular confirmation should be viewed

with caution (329). Nonetheless, CHALKER *et al.* proposed a new human HM species: '*C*M haemohominis' (41).

II.2.3.9 Further HM species/ isolates

Novel HM species were described in racoon (*H. procyoni*) (90), opossum ('*C*M haemodidelphis') (207, 208), flying fox (*E. mariboi*) (76), sea lion ('*C*M haemozalophi') (351), and sika deer ('*C*M haemocervae', '*C*M erythrocervae') (355). In reindeer (304), HM-like organisms were found, but the species was not identified.

II.2.3.10 Situation in horses

Previously, little was known about possible HM infections in horses. Initial observations of possible infection can be traced back to 1978, when GRETILLAT reported a putative 'haemobartonellosis' outbreak in Nigeria (101). Horses showed the following clinical signs: general weakness, hyperthermia, inappetence, dyspnoea, anoxia, pale mucous membranes, profuse sweating after some minutes of trot, petechia, mild jaundice, epistaxis after exercise, joint and muscle pain, stumbling, and painful and swollen lymph nodes. Severe manifestations were observed in horses that were already stressed. HM-like particles of approx. size of 0.3 µm diameter were found on the surface of erythrocytes in May-Grünwald and Giemsa-stained peripheral blood smears (101). After this first indication of HM infection in horses in 1978, nothing about this kind of infection in horses was reported until 2001, when TARELLO published a case report about the 'equine fatigue syndrome' in combination with detection of small coccoid eperythrocytic bacteria (318). The nature of these bacteria, however, was not elucidated. Later, a puative HM infection of German horses was reported based on cytology (93, 230, 231). The syndrome reported by TARELLO resembled the previously reported unspecific clinial signs, signifying possible HM infection in other animal species. In all four of these caes, suspected diagnosis was only made by monitoring unspecific clinical signs and a miroscopic appearance resembling HM infection. Proof of HM infections in horses by molecular diagnostic techniques remains to be seen.

II.2.4 Transmission

Exact transmission pathways for natural HM infections remain unclear, although several transmission routes have been proposed in scientific literature.

II.2.4.1 Blood-sucking arthropods

Blood-sucking arthropods involved in transmission of HM may include fleas, hard ticks, and mosquitoes (220), as in the case of rickettsia and spiroplasmas (141, 264, 301). *Hyalomma anatolicum* possibly transmits *M. wenyonii* (162). *M. haemocanis* may be transmitted by the tick *Rhipicephalus (R.) sanguineus* (283), *M. haemomuris* via the mouse louse *Polyplax (P.) spinulosa* and the flea *Xenosylla cheopis* (22), and *M. coccoides* via the mouse lice *P. spinulosa* (74) and *P. serrata* (22). The rat louse *P. spinulosa* was also proposed as vector for '*C*M haemominutum' (75, 220). Transmission of *M. suis* via flies (*Stomoxys (S.) calcitrans*), mosquitoes (*Aedes (Ae.) aegypti*) and lice (*Haematopinus suis*) was proposed (253, 291, 292). *M. ovis* may be transmitted via ticks (*Haemaphysalis plumbeum*, *R. bursa*) (232), mosquitoes (*Ae. camptorhynchus*, *Culex annulirostris*, *Anopheles annulipes*) (54, 142, 228), stable flies (*S. calcitrans*) (239), and keds (*Melophagus (Me.) ovinus*) (239). Besides ticks, *M. haemofelis* and '*C*M haemominutum' may be transmitted by the cat flea *Ctenocephalides felis* (160, 177, 284, 363), even though experimental transmission studies were not conclusive (372).

Different arthropod species may vary in capability to harbour and transmit HM. MASON & STATHAM showed that stable flies would be more effective vectors than ticks, as ticks only transmit one or two RBCs, while stable flies can transmit up to 400 RBCs (197).

Transmission via haematophagous arthropods is corroborated by findings of geographical variations in HM infection prevalence (95, 165, 310, 358, 361). Incidences of canine HM are higher in countries with higher mean annual temperatures, where *R. sanguineus* is endemic (11, 23, 165, 283). Therefore, the frequency of infection may be hypothetically related to the abundance of the relevant transmission vectors.

'*C*M haemominutum' was found in unfed *Ixodes (I.) ovatus* ticks in Japan (319) and *M. haemofelis* in *I. ricinus* (273). However, a large study analysing almost 2000 ticks in Switzerland revealed no HM positive results in unfed *Ixodes* sp., but in some *Ixodes* sp. and *Rhipicephalus* sp. which were directly collected from animals (363). OVERAS (239) and FOOGIE & NISBET (87) infected sheep with the stable fly (*S. calcitrans*) and head lice (*Linognathus ovillas*) resulting in HM infection in sheep, but transmission under natural conditions was not shown. Transmission via removed sheep keds (*Me. ovinus*)

from HM infected sheep and injection into healthy sheep did not result in visible infection (87, 226).

Presence of HM in wild animals (108, 128, 350, 351, 355, 365) lead to the assumption that wild species may serve as reservoir hosts.

II.2.4.2 Direct transmission

In cats, direct transmission via bites and scratches during fighting seems to govern natural infection. Feline HMs were detected in saliva, salivary glands, gingival, claw beds, and faeces of infected cats (58, 105, 215, 360, 363). This hypothesis is substantiated by the higher prevalence of HM infection in male cats, cats with outdoor access, and cats with bite abscesses (105, 271, 325, 361). However, oronasal and subcutaneous transmission of '*C*M turicensis' via saliva and oral transmission of blood failed, while subcutaneous exposure of '*C*M turicensis' infected blood resulted in PCR positive animals, even though overt clinical signs were absent (215). Similar results have been found for canine HM (164, 236).

II.2.4.3 Vertical transmission

First indications of *in utero* or lactogenic transmission was reported for pigs (24, 121), sheep (104), cats (114), and llamas (6, 81). YANG *et al.* detected HM in cord blood of human newborns (373). However, the hypothesis of vertical transmission has not yet been systematically proven.

II.2.4.4 Iatrogenic transmission

Iatrogenic transmission via contaminated needles, surgical instruments, blood transfusion, tattoo needles, ear tagging, tail docking, and shearing frequently occur (6, 38, 64, 122, 157, 179, 210, 223, 305). Also transmission of bovine HM via automatically rotating brushes may be possible (139).

M. suis and *M. haemofelis* can be experimentally transmitted via subcutaneous, intravenous, intraperitoneal, and oral inoculation (83, 136).

II.2.5 Diagnostics

II.2.5.1 Microscopy

Since molecular diagnostic tools are a rather recent invention and HM are not cultivable, HM diagnosis was previously performed with microscopic evaluation of peripheral blood smears stained with Romanowsky, Wright or Giemsa staining solution. They are based on a differential blood staining principal: various blood cells exhibit different staining properties based on the pH during staining procedure (28, 274).

Staining with acridine orange can improve sensitivity but a fluorescence microscope is required for evaluation (28, 109, 288, 321).

Improvement of diagnostic methods revealed the shortfalls of these staining methods, poor sensitivity (less than 20 %) and specificity (133, 154, 182, 321, 359). Staining precipitates, Heinz-, Pappenheimer-, and Howell-Jolly bodies can be confused with HM (114, 239, 321), and species differentiation is virtually impossible (321). Further, for '*C*M turicensis' it was reported that the blood loads were too low for detection of HM on the surface of RBCs (361, 367). A recent discovery showed that bacteria can detach from RBCs in EDTA anti-coagulated blood, leading to an underestimation of parasitaemia (5).

II.2.5.2 Splenectomy

Parasitaemia of RBCs only occurs during the acute phase of infection. Usually, chronic infections cannot be diagnosed via microscopy (5, 118, 209, 239). Before DNA extraction and PCR methods were available, animals suspected of asymptomatic or chronic course of disease are forced to undergo splenectomy. If the animal becomes anaemic, and HM could be revealed on RBCs in peripheral blood smears, a HM infection was confirmed (118, 210). An alternative, less ethically sound method of diagnosis, is to transmit suspected animals' blood to susceptible animals. For HM-positive animals, subsequent infection produces anaemia, and a parasitaemia of RBCs can be observed (34, 136, 275, 332, 333).

II.2.5.3 Polymerase chain reaction (PCR)

In the 1990s, the first conventional PCR diagnostic assays specific to HM were developed based on the 16S rRNA gene: *M. suis* (206), *M. haemocanis* (32, 358), feline HM (49, 154, 205), and *M. haemomuris* (378). Also, assays based on other genes were published, e.g. on a *M. suis* specific 1.8 kb genomic fragment (131) or the *rpoB* gene (111).

Up to now, PCR is the "method of choice" (364), as it is well-suitable for detection of HM in blood of acute, latently infected, and asymptomatic animals (136).

PCR diagnostics were greatly improved by the development of quantitative real-time PCR assays, which improved the speed, ease, and sensitivity of HM diagnosis (133, 182, 190, 201, 325, 358). No elaborate post-PCR steps are required anymore (190). Fluorescent labelling of primer (TaqMan system) or amplified DNA (SYBR green I system) enable visualisation in real-time (190). This technique exhibits low contamination risk due to a closed tube system, allows standardisation, and is easily reproducible (136, 190, 364). Automation facilitates high throughput in daily routine diagnostics and makes prevalence studies possible.

However, specific PCR primers can only be designed if the sequence or part of a sequence of at least one gene is known. 16S rRNA, for example, is well-suited for comparative sequence analysis due to its hyper-variable and conserved regions across all eubacteria. Universal 16S rRNA oligonucleotides are available which amplify the 16S rRNA gene of most eubacteria. WILLI *et al.* reported the development of a SYBR green I real-time PCR assay which is suitable for detection of all known HM species (366). This assay may be useful to screen for hitherto unknown HM species in animal blood samples. Amplicons may be sequenced for further analysis and species identification.

II.2.5.4 Serology

Serological methods enable screening of a high sampling number at low costs. This is crucial for e.g. livestock husbandries who wish to affordably screen their populations. However, development of serological detection methods is hindered by unculturability of HM (136). As antigens are derived from experimentally infected animals, purification is very elaborate, and preparations may contain host antigens. Nevertheless, six immunogenic proteins were identified for *M. suis*: HspA1, GroEL, enolase, pyruvate dehydrogenase, RNA helicase, and actin-analogous protein (132, 134, 136), and several testing methods were established for *M. suis* diagnosis: (i) a complement fixation assay (CFA; 10, 279, 300), (ii) an indirect haemagglutination assay (IHA; 290), and (iii) an enzyme-linked immune assay (ELISA; 126, 145, 279). For *M. ovis*, (i) an indirect immunofluorescent antibody test (IFAT; 146, 149, 229), (ii) a CFA (50), and (iii) an ELISA (176) were reported. A Western immunoblot antibody test for feline HM was published (5), and WOLF-JACKEL *et al*. described a recombinant antigen for serological investigation of feline HMs (370).

II.2.6 Therapy

Since mycoplasmas lack cell walls, they are resistant to penicillin and its derivates but susceptible to tetracyclines and other antibiotics which are not targeted against the cell wall, e.g. inhibitors of the gyrase (220).

Pigs were traditionally treated with tetracyclines (oxytetracycline, doxycycline) and arsenic derivatives (diminazen aceturate). Auxiliary treatment of clinical signs by administration of glucose and iron dextran is helpful in therapy of *M. suis* infections. However, complete elimination of bacteria from blood by use of tetracyclines is not possible (100, 122, 136).

In sheep, treatment with tetracyclines (oxytetracycline, chlortetracycline) and arsenic derivatives leads to a reduction of bacteria in blood but not to a complete elimination (92, 227). Using imidocarb dipropionate, *M. ovis* may be completely removed, although recrudescence of infection and race-dependent side-effects may be observed (147). A supplementary diet was prescribed to improve general health condition of infected sheep (36).

The three feline HM species vary in their therapeutic susceptibility. No therapy tested thus far (tetracycline: doxycyline; fluoroquinolon: enrofloxacin, marbofloxacin) was able to completely eliminate bacteria from blood of infected cats, but severity of clinical signs and bacterial blood loads were reduced (20, 68, 85, 209, 321, 326, 327, 361). Azithromycin was not effective (359). In severe cases of anaemia, a blood transfusion may be indicated (321). In some *M. haemofelis* infections, administration of glucocorticoids (e.g. prednisolone) may be required, but interfering infections should be ruled out (321).

Dogs with an HM infection are treated with oxytetracycline or doxycycline (42, 209).

In llamas, tetracycline therapy lead to health improvements, but bacteria was not completely eliminated from blood, resulting in an asymptomatic carrier state (336).

The best ‘therapy’ remains the prevention of blood transmission in animal husbandries by better hygiene, control of endo- and ectoparasites (36), removal of asymptomatic carrier animals (PCR testing), and screening of blood donor animals (209, 361).

No vaccine is available, although immunogenic proteins of *M. suis* have been described (127, 134). Health condition and immune status can be improved by supplementation of e.g. iron and copper (92). GRETILLAT also reported in 1978, that horses having access to good quality feed showed less severe manifestations of ‘haemobartonellosis’ (101).

II.2.7 Taxonomy of haemotrophic mycoplasmas

The first representatives of the haemotrophic mycoplasmas (HM) were found in the beginning of the 21st century and were classified as members of the genus *Bartonella* (167), *Haemobartonella* (342), or *Eperythrozoon* (275). Since then, several anaemia inducing parasites of mammalian RBCs were found (Tab. II-1, pp. 9-10) and sorted into the genera *Haemobartonella* or *Eperythrozoon*, respectively. Species names were given according to host, as strict host specificity was assumed (220). In the pre-sequencing era, bacteria were classified using morphological and infectious criteria. Due to their small size, gram-negative staining features, and suspected transmission via blood-sucking arthropods, HMs were originally classified within the group of *Anaplasmataceae* in the class of *Rickettsia* (268). Differentiation of *Haemobartonella* and *Eperythrozoon* was somewhat arbitrary, as it relies solely on morphological and infectious criteria. While *Eperythrozoon* species frequently form ring forms and are more often found free in the plasma, *Haemobartonella* species do not assume a ring form and occur only rarely free in the plasma (210, 220). Electron microscopy studies revealed that the fine ultrastructures of both genera are fundamentally similar. Cross-infection studies were performed to discriminate HM species from each other (52, 144, 172, 189, 199).

In 1965, TANAKA *et al.* first proposed that these two genera may belong to the class of *Mollicutes* because they share important characteristics like unculturability, lack of intracellularity, lack of a cell wall and flagellae, and resistance to penicillin but susceptibility to tetracyclines (316). When the first 16S rRNA sequences of *Haemobartonella* sp. and *Eperythrozoon* sp. were available in the 1990s, their closer relationship to the genus *Mycoplasma* within the group of *Mollicutes* was proven. Reclassification of *Haemobartonella* and *Eperythrozoon* species as *Mycoplasma* species was then suggested (208, 219, 220, 222, 224, 265).

The class of *Mollicutes* is grouped within the clostridial subdivision of the gram-positive bacteria exhibiting a low GC % content and is phylogenetically diverse, comprising an increasing number (currently more than 180) of species in eight genera (*Mycoplasma*, *Ureaplasma*, *Spiroplasma*, *Acholeplasma*, *Anaeroplasma*, *Asteroplasma*, *Mesoplasma*, and *Entomoplasma*) (218, 258, 261, 338, 368). One of the most striking features of *Mollicutes* is the absence of a cell wall. Mycoplasmas are the most rapidly evolving bacterial lineage, and they show an unusual mode of evolution, the so called “degenerative evolution” (369), descending from *Clostridia*. Through reductive evolution, they have lost many genes. Today, they represent bacteria with the smallest reported ge-

nomes, consisting of essential genes only (217, 218, 225, 357, 368). An alternative hypothesis suggested that mycoplasmas are descendants of primitive ancestral cells that existed before the development of cell walls and represent one of the most primordial organisms (258). However, the aforementioned "degenerative evolution" hypothesis has been supported by rRNA and tRNA sequencing showing structures which more closely resemble gram-positive than gram-negative bacteria (218, 258, 368). For a detailed review concerning the phylogenetic theories of *Mollicutes*, refer to RAZIN (258).

The HMs form a distinct cluster within the genus *Mycoplasma* (219, 265), which is most closely related to the 'fastidiosum-cluster' (*M. fastidiosum*, horse; *M. cavipharyngis*, guinea pig) (155). Both clusters belong to the 'pneumoniae-group', which includes the human pathogens *M. pneumoniae*, *M. penetrans*, and *M. genitalium* (155, 208, 219, 220, 357).

Analysis of additional phylogenetic marker genes (e.g. *rnpB*) confirmed the proposed classification and close relationship to the 'pneumoniae-group' (243, 324), although differences with the previously published *Mycoplasma* classifications were found (324, 357). Analysis of the *rnpB* gene afforded better resolution of closely related species, as it shows a higher rate of variation (317). *M. haemocanis* and *M. haemofelis* cannot be distinguished by 16S rRNA sequencing, but *rnpB* sequences revealed two distinct clusters (26, 324).

Two groups were able to be differentiated within the HM cluster based on 16S rRNA and *rnpB* sequence analyses: 'haemofelis'- und 'haemominutum-group' (243). A characteristic truncation of 10 bp (position 453-481, *E. coli* numbering) was found in the 16S rRNA sequence of members of the 'haemofelis-cluster' (155, 208). WILLI speculated that HM species from different clusters may differ in pathogenicity (364). However, this requires further study.

UILENBERG *et al.* have questioned the reclassification of HM, because HM and non-HM show higher 16S rRNA identities than members of well-defined genera such as *Staphylococcus* and *Streptococcus* or *Clostridium* and *Bacillus* (346, 347). Additionally, HM exhibit unique properties (habitat, uncultivable, and suspected vector transmission). That is why UILENBERG *et al.* suggested that HM (*Eperythrozoon* as well as *Haemobartonella* sp.) should be placed in their own genus named *Eperythrozoon* beside the genus *Mycoplasma* (including the non-haemotrophic *Mycoplasma* sp.) within the family of *Mycoplasmataceae* within the genus *Mollicutes* (346, 347). He pointed out that the transfer of *Eperythrozoon* species to *Mycoplasma* species does not follow the rules of

nomenclature, as the genus name *Eperythrozoon* was assigned earlier (1928) than the genus *Mycoplasma* (1929) (224, 237, 275, 346, 347). Though the reclassification would violate naming conventions. However, exceptions can be made "where strict adherence to the rules of nomenclature would produce confusion or would not result in nomenclatural stability" (Judical Commission of the International Committee on Systematics of Prokaryotes, Appendix 8). An exception was requested by NEIMARK *et al.* (224).

II.3 Anaemia in Horses

II.3.1 Anaemia

Anaemia is characterised by decreased mass of RBCs, low haematocrit, and/or decreased haemoglobin concentrations leading to diminished oxygen transport capabilities. Clinical signs of anaemia may include pallor of mucous membranes, exercise intolerance, weight loss, weakness, fatigue, rapid exhaustion, dyspnoea on exertion, tachypnea, tachycardia, mitral valve murmur, hypoxia, haematuria, proteinuria, and icterus. Equine anaemia occurs concurrently with certain diseases or pathological changes, including blood loss, increased RBC destruction, and inadequate RBC production. Horses do not release reticulocytes or other juvenile forms of RBCs into the bloodstream. Thus differentiation of regenerative (in case of blood loss and haemolysis) and non-regenerative anaemia (in case of malfunctioning RBC production) is hampered. Anaemia can be categorised according to RBC morphology, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) (80, 94, 178, 213, 341).

However, changes in erythrocytic indices are rare in horses, and mostly a <u>normochromic, normocytic anaemia</u> is diagnosed (80, 175). In this case, RBCC is lowered, but cells appear morphologically normal. Anisocytosis and polychromasia are rarely observed in cases of equine anaemia (213). Either RBC production is reduced (e.g. chronic inflammatory or neoplastic diseases) or RBC degradation is increased (e.g. haemolytic anaemia). In normochromic, normocytic anaemia, MCV, MCH, and MCHC remain at normal levels (80, 175, 178, 341).

<u>Hyperchromic and macrocytic anaemia</u> result from a deficiency in certain vitamins (e.g. vitamin B_{12}, folic acid) and result in restricted production of RBCs. Since these vitamins are needed for DNA synthesis, there is not enough DNA for cell division, and RBCs do not divide and remain as enlarged cells (megaloblasts). However, vitamin B_{12} and/or fo-

lic acid deficiencies are very rare in horses. MCV is increased and MCH and MCHC are decreased (175).

Hypochromic and microcytic anaemia can be induced by iron deficiencies, when cells undergo additional cell division due to low haemoglobin concentrations. Iron deficiency, however, does virtually not occur in horses, since adult horses have sufficient iron stored in tissues. MCV, MCH, and MCHC are decreased, while RBCs are smaller and show a greater central pallor. Deficiency may be caused by increased loss of iron due to unrecognised chronic bleedings, increased haemolysis, or insufficient absorption. Also, this type of anaemia may be induced by chronic diseases, immune mediated diseases, and acute or chronic infections (175, 178, 341).

In horses, haemorrhage and haemolytic anaemia have the most important clinical significance (175).

II.3.2 Haemolytic anaemia

Haemolytic anaemia is characterised by increased intra- and/or extravascular destruction of RBCs before they reach their physiological age of three to five months, when RBC destruction is not compensated by newly produced RBCs. Poikilocytosis is frequently observed due to increased RBC fragility. RBCs can be damaged by corpuscular (e.g. defective RBC membrane, haemoglobin molecules, or enzymes) or extracorpuscular (e.g. microangiopathetic processes, drugs, toxins, immune mediated diseases, and damages due to parasites, bacteria, and viruses) reasons (80, 175, 178, 213, 330, 341).

Due to destruction of RBCs, plasma levels of potassium, unconjugated bilirubin, and iron are increased. Increasing bilirubin concentrations in blood may lead to icterus. Also haemoglobinuria and proteinuria may occur. MCH and MCHC are usually increased due to elevated free haemoglobin levels.

In autoimmune haemolytic anaemia, RBC destruction is initiated by binding of autochthonous IgG and/or IgM antibodies to the surface of RBCs. In the first case, IgG antibodies attach to the surfaces of RBCs, thereby removing RBCs from circulation, resulting in extravascular haemolysis. These antibodies exhibit the strongest reactivity close to 37 °C. Haemograms of warm autoimmune haemolytic anaemia often show variable haemoglobin and haematocrit values but elevated MCV and WBCCs (94, 341).

In the second case, RBCs agglutinate due to attachment of so-called cold agglutinins (IgM auto-antibodies) at low temperatures. This attachment leads to acrocyanosis, where haemolysis is induced via binding of cold auto-antibodies in the cooler peripheral circulation (94, 183, 341). Cold agglutinins by definition exhibit their strongest reactivi-

ty at temperatures near 4 °C and show decreased binding at higher temperatures. IgM-induced haemolysis can occur intra- or extravascularly (94). Cold autoimmune haemolytic anaemia can be induced idiopathically or as a result of *Mycoplasma* infections (e.g. *M. suis* in pigs (138, 377), *M. pneumoniae* in humans (43, 77)). In haemograms, slightly decreased haemoglobin and haematocrit values will be detected, while the leukocyte and platelet numbers are usually normal. Due to RBC clumping, the MCV can be artificially increased, while the apparent RBC count is decreased (94).

Additionally, a mixed-type (both IgG and IgM bind to the RBCs) and a drug-induced immune haemolytic anaemia (e.g. due to high-dose penicillin, N-propyl disulfide of red maple leaf) are known (94).

II.3.3 Anaemia inducing diseases and infections

Anaemia may be induced due to vitamin deficiencies (e.g. iron, folic acid, or vitamin B_{12}), certain diseases and pathological changes (e.g. immune mediated diseases, infections, or tumours), or intoxications (e.g. drugs or red maple leaf) (27, 80, 175, 178, 213, 330, 331, 341).

Equine infectious anaemia (EIA) is caused by a virus, which is passed on by blood-sucking arthropods. Since these insects prefer warm and humid regions, EIA is not endemic in Germany. In cold regions, infections can be imported through foreign horses or horses with travel history in endemic regions. Viruses attach to RBCs and induce an immune-mediated haemolysis leading to severe anaemia. Infection is accompanied by neutropenia and lymphocytosis (282, 330).

Babesia caballi and *Ba. equi* (synonym: *Theileria equi*; equine piroplasmosis) are protozoan parasitizing equine RBCs transmitted by ticks. Although a small number of RBCs may be infected, *Babesia caballi* can lead to severe anaemia (178). The disease is not endemic in Germany but is common in North- and South America and parts of East- and South Europe (330).

Ehrlichia equi is a bacterium belonging to the class of *Rickettsia*, which parasites in leucocytes and is presumably transmitted by ticks. Although their haemograms exhibit only unspecific changes and clinical sings resembling EIA, ehrlichiosis can be clearly diagnosed due to typical morula-like inclusions in the cytoplasm of eosinophils and basophils. Only sporadic cases of ehrlichiosis are reported in Germany (330).

Rarely, haemolysins of *Staphylococcus* sp., *Clostridium* sp., or *Leptospira* sp. can induce haemolytic anaemia in horses (330).

III. GOAL OF THIS THESIS

III. GOAL OF THIS THESIS

III.1 HM in horses

In Northern Germany, especially in the region of the Luneburg Heath, several horse owners reported horses showing inhibited development, apathy, shaggy fur, and poor overall health. These unspecific clinical signs were sometimes accompanied by slight anaemia. Thorough clinical examinations revealed no cause for these unspecific clinical signs, and other infective agents resulting in anaemia (e.g. *Theileria* sp., *Babesia* sp., or EIA virus) were determined not to be the underlying cause. In peripheral blood smears, HM-like particles of an approx. size of 0.3-0.4 µm were observed (DR. MICHAEL DIECKMANN & DR. MARGIT WINKLER, pers. comm.).

The goals of this thesis research were to:

(i) prove by use of molecular diagnostic techniques that HMs infect horses,
(ii) develop a specific diagnostic tool,
(iii) investigate the clinical significance of this type of infection in horses, and
(iv) further characterise this novel infective agent.

III.2 Phylogenetic analysis of novel HM isolates

In the second part of this thesis, the current HM classification was re-evaluated and novel HM isolates from horses, cattle, wild boars, and llamas were classified and their phylogenetic position clarified.

IV. MATERIAL & METHODS

IV. MATERIAL & METHODS

IV.1 Blood sample collection

IV.1.1 Blood sample collection of horses

Blood samples were collected from horses' *vena jugularis externa* (Vacuette Greiner Bio-One tube system) and anti-coagulated with EDTA, Citrate, or Alsever's solution. Figure IV-1 and Table IV-1 summarise the horse blood samples collected during this study; a detailed overview is given in the appendix (Tab. VIII-1, pp. 165-175; also compare DIECKMANN *et al.*, 2010 (62) & 2011 (63); pp. 134-136 & 140-164). All horses originated from the Luneburg Heath (Northern Germany) without travelling history, if not indicated otherwise.

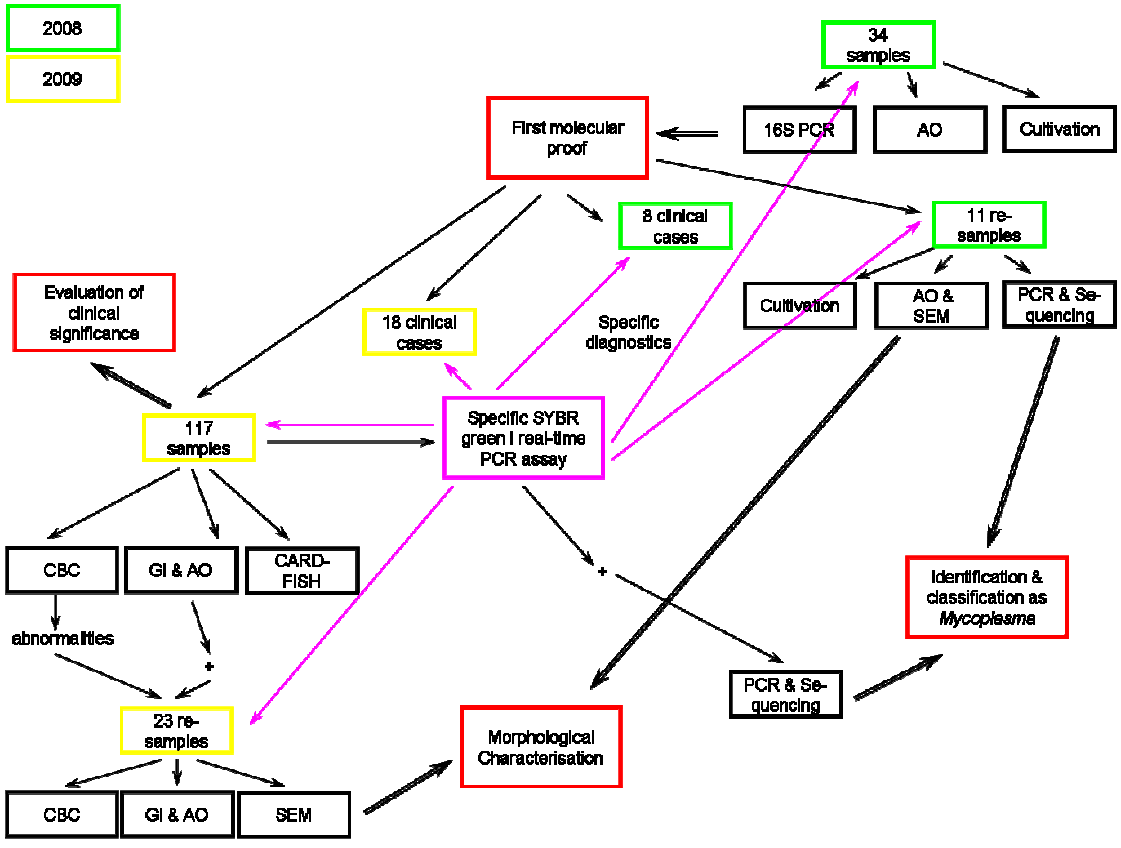


Figure IV-1 Workflow of sample procession. Initially, 34 samples were collected for molecular proof of the existence of HM in horses in 2008; samples were analysed using PCR and acridine orange stained peripheral blood smears (AO). After the successful proof, eleven samples were re-collected for morphological characterisation due to scanning electron microscopy (SEM). In 2009, 117 samples were collected to evaluate the clinical significance of equine HM infections; complete blood counts (CBC) were prepared, and samples were analysed by PCR and microscopy of Giemsa (GI) and acridine orange stained peripheral blood smears. HM positive samples were used to establish a SYBR green I real-time PCR assay specific to the novel equine HM isolate. This assay was then used for specific diagnostics (purple arrowheads).

Table IV-1 Summary of horse blood samples (n = 283) analysed during this study

Time period	Number	Origin of samples
Spring 2008	34	Luneburg Heath, Northern Germany; MD
Summer 2008	11	Luneburg Heath, Northern Germany; MD
Autumn/Winter 2008	8	Luneburg Heath, Northern Germany; MD
November 2009	117	Luneburg Heath, Northern Germany; MD
December 2009	23	Luneburg Heath, Northern Germany; MD
Autumn 2008 & 2009	72	Switzerland; Negative controls
Winter 2009/2010	18	synlab.vet, Geesthacht, Northern Germany

MD = Samples collected by DR. MICHAEL DIECKMANN.

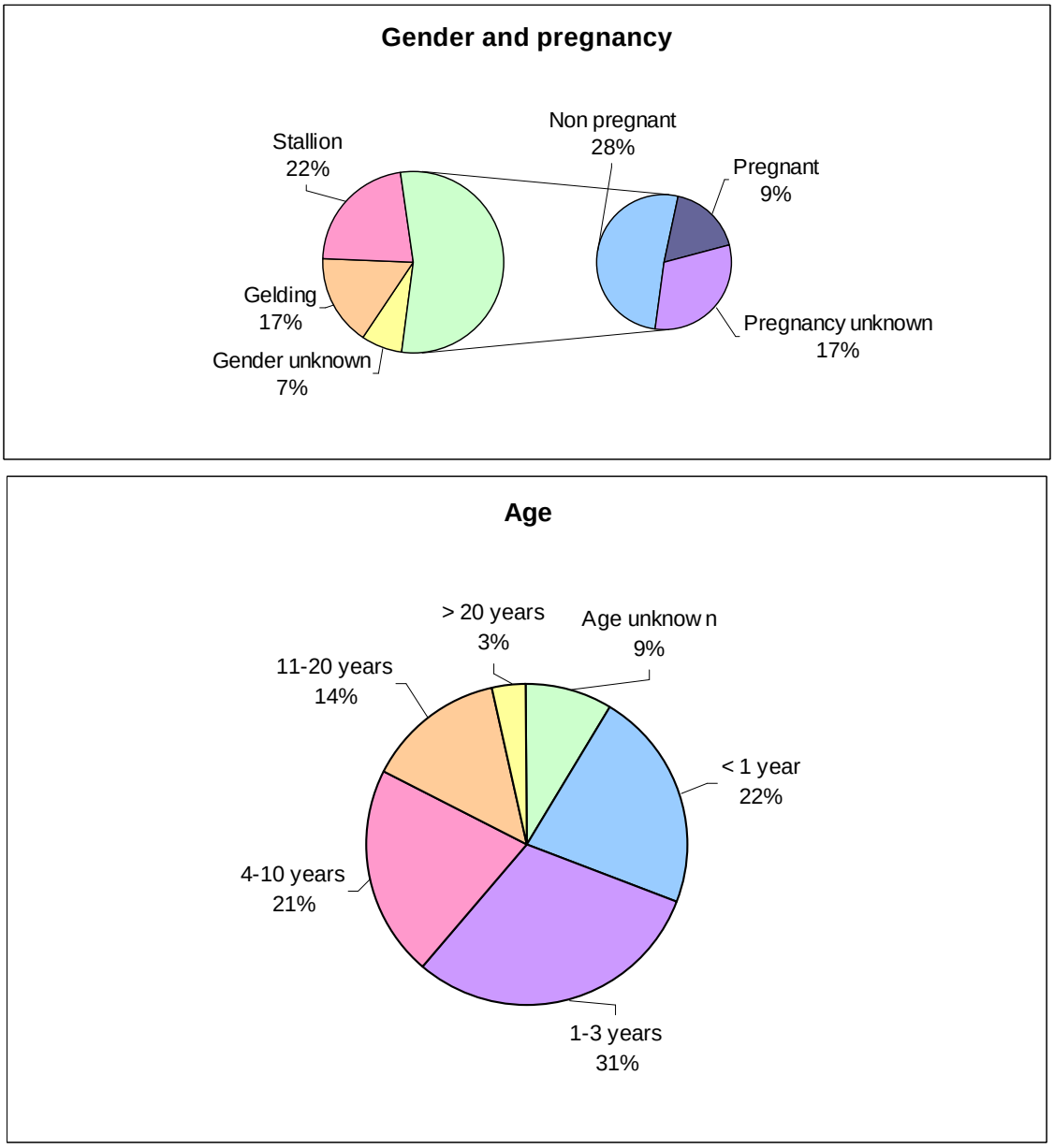


Figure IV-2 Characteristics of horse samples (n = 211) collected during this study. Distribution of gender, pregnancy status and age is outlined. Horses from the negative collective are not shown.

Blood of 211 horses was analysed: 114 mares (54.0 %), 35 geldings (16.6 %), and 47 stallions (22.3 %) were included. Fifteen horses' gender was not known (7.1 %). The mean age was 5.7 years (range: nine months to 26 years). 175 of the horses were warm blood breeds (82.9 %; mainly Hanoverian (70.1 %)). Further, there were four German riding ponies (1.9 %), one Shetland pony (0.5 %), one tinker (0.5 %), three Friesian (1.4 %), one thoroughbred (0.9 %), one Arabian (0.5 %), and one Pinto (0.5 %) included into the study. Twenty-four horses' breed was unknown (11.4 %) (Fig. IV-2, p. 31).
To establish a negative control group, 72 blood samples from healthy Swiss horses were included (Tab. IV-I (p. 31) & VIII-2 (pp. 176-179)). These samples were tested negative for any HM infection by a SYBR green I real-time PCR assay comprising all known HM species (366). The mean age of the negative horse collective was 12.1 year (range: nine months to 39 years). Twenty-eight females (38.9 %) and 40 males (55.6 %) were included. Gender of four horses and pregnancy status were unknown. Twenty-eight warmblood horses (38.9 %), five Draft (6.9 %) horses, four Thoroughbreds (5.6 %), twenty ponies (27.8 %), and nine horses of various breeds (12.5 %) were included. Six horses' breed (8.3 %) was unknown.
From selected horses a complete blood count was prepared (synlab.vet) (Tab. VIII-1, 2 & 3; pp. 165-187).

IV.1.2 Blood sample collection of cattle

For details about the collection procedure see HOELZLE *et al.*, 2011 (130); pp. 137-139). Blood was collected from twenty animals showing the following clinical signs: anaemia, decreased milk production, infertility, and lameness.

IV.1.3 Blood sample collection of wild boars

A total of 359 EDTA-anti-coagulated blood samples from wild boars were included. Animals were hunted in the time period from December 2007 to January 2008 in South Germany. For details see HOELZLE *et al.*, 2010 ((128); pp. 129-133).

Table IV-2 Composition, preparation, and cultivation condition of used culture media

Medium	Gassner agar	Colombia blood agar	Chocolate agar	LB medium	LBZ agar
Composition/ preparation				37 g LB broth, ad 1 L H_2O_{MQ}; autoclaving: 15 min, 121 °C	37g LB agar, ad 1 L H_2O_{MQ}; autoclaving: 15 min, 121 °C; chilling to 50 °C; 1 mL ampicillin (100 mg/mL)[a], 1 mL X-Gal (80 mg/mL)[b], 1 mL IPTG (1 mmol/L)[a]
Reference	Oxoid AG, Pratteln, CH				
Cultivation	37 °C, aerobic, 24 h	37 °C, 5 % CO_2, 48 h		37 °C, aerobic, 24 h	37 °C, aerobic, 24 h

Medium	PPLO agar	PPLO broth	SP4 broth	SP4 agar	modified RPMI broth
Composition/ preparation	10.5 g PPLO agar, 195 mL H_2O_{MQ}; autoclaving: 15 min, 121 °C, 75 mL horse serum[c] 30 mL yeast extract (with K_2PO_4), 1 mL thallium acetate (10 %), 2.25 mL NAD solution (1 %), 0.75 mL penicillin[d]	10.5 g PPLO broth, 195 mL H_2O_{MQ}; autoclaving: 15 min, 121 °C, 75 mL horse serum[c] 30 mL yeast extract (with K_2PO_4), 1 mL thallium acetate (10 %), 2.25 mL NAD solution (1 %), 0.75 mL penicillin (final concentration 500 U/mL)[d]	11 g PPLO broth, 10 g tryptone, 5 g glucose, 625 mL H_2O_{MQ}; adjust pH: 7.5; autoclaving: 15 min, 121 °C; 50 mL mycoplasma growth supplement (10x, CMRL 1066), 35 mL yeast extract solution[e] 100 mL yeastolate (2 %), 170 mL foetal bovine serum[c]	11 g PPLO agar, 10 g tryptone, 5 g glucose, 625 mL H_2O_{MQ}; adjust pH: 7.5; autoclaving: 15 min, 121 °C; 50 mL mycoplasma growth supplement (10x, CMRL 1066), 35 mL yeast extract solution[e], 100 mL yeastolate (2 %), 170 mL foetal bovine serum[c]	500 mL RPMI 1640 (with L-glutamine), 5 mL HEPES buffer (1 M), 5 mL sodium pyruvate (100 mM), 5 mL non-essential amino acids (100x), 1 mL haemin (7.5 mg/mL)[f]; adjust pH: 7.0
Reference	73		ATCC medium 988		371
Cultivation	37 °C, 5 % CO_2, up to six weeks				

All media were sterile filtered with a pore size of 0.2 µm; medium components were purchased from Difco or Gibco, [a] ampicillin (Sigma-Aldrich) and IPTG (Chemie Brunschwig AG) were solved in H_2O_{MQ}; [b] X-Gal (Chemie Brunschwig AG) was solved in DMF; [c] heat-inactivated at 56 °C for 1 h; [d] Hoechst; [e] 250 g Baker's yeast, ad 1 L H_2O_{MQ}; [f] solved in 0.01 M NaOH; ATCC = American Type Strain and Culture Collection

IV.2 Cultivation

Gassner-, Colombia-blood-, chocolate-, SP4-, and PPLO-agar plates as well as PPLO-, SP4-, and modified RPMI-broth were each inoculated with 10 µL EDTA-anti-coagulated horse blood and incubated, as outlined in Table IV-2 (p. 33). Additionally, blood culture systems (Oxoid) were inoculated with 10 mL of fresh horse blood and incubated for six weeks at 37 °C. Liquid cultures and blood cultures were checked weekly for microbial growth due to subcultivation on Gassner-, Colombia-blood-, chocolate, SP4-, and PPLO agar plates, in addition to PCR targeting universal regions of the 16S rRNA gene (Ch. IV.6.2., p. 38) and microscopy of Gram- and Giemsa-stained samples (Ch. IV.3, p. 34).

Transformed *Escherichia coli* cells were grown on LBZ agar plates (Tab. IV-2; Ch. IV.7, p. 40).

For longer storage of bacterial cultures, glycerol stocks were prepared. 1 mL mid-log culture was mixed with 1 mL glycerol solution (65 % glycerol (v/v), 0.1 M $MgSO_4$, 0.025 M Tris (pH 8.0)), frozen rapidly and stored at -80 °C.

IV.3 Microscopy

IV.3.1 Optical microscopy

IV.3.1.1 Preparation of peripheral blood smears

Peripheral blood smears were produced using the wedge method, as described by LATIMER & RAKICH (178). One drop of blood was placed on a glass slide (SuperFrost, Menzel), and a second slide was drawn backwards into the drop of blood. Then, the spreader slide was rapidly and smoothly moved forward before air-drying.

IV.3.1.2 Giemsa staining

Peripheral blood smears were fixed in methanol (Roth) for 5 min and incubated for 2 h in Giemsa staining solution (190 mL H_2O_{MQ} (buffered by Sörensen phosphate buffer (Merck)), 10 mL Giemsa solution (Merck), incubation for 10 min at RT, sterile filtration (pore size 0.2 µm)). The slides were washed twice for 1 min in buffered H_2O_{MQ} and air-dried.

IV.3.1.3 Acridine Orange staining

Peripheral blood smears on glass slides (SuperFrost, Menzel) were fixed in $EtOH_{100\,\%}$ (Fluka) for 1 min, air-dried, and incubated in acridine orange staining solution (0.02 %) for 1 h in the dark. Afterwards, the slides were rinsed with $Aqua_{bidest}$ and air-dried. Stained samples were analysed using fluorescence microscopy (Olympus BX50).

IV.3.1.4 Gram staining

Samples were spread on glass slides (SuperFrost, Menzel), fixed by heat, and stained with crystal violet (R1; BioMérieux) for 3 min at RT. The staining solution was discarded; slides were stained with lugol solution (R2; BioMérieux) for 2 min at RT, and then rinsed with $Aqua_{bidest}$. After decolourisation in $EtOH_{100\,\%}$ (Fluka) until blue clouds were no longer washed away, the slides were rinsed with $Aqua_{bidest}$ and stained in safranin (R3; BioMérieux) for 30 s.

IV.3.2 Scanning electron microscopy

For fixation, 100 µL of fresh anti-coagulated blood was mixed with 2.5 mL PBS-GB (1x PBS, 10 mM glucose, 0.1 % BSA (Sigma-Aldrich)) and incubated at 37 °C for 15 min. Slowly, 2.5 mL PFA-GA (1x PBS, 4 % PFA (Sigma-Aldrich), 0.01 % GA (Sigma-Aldrich)) were added drop-wise. Occasionally, the tube was closed and carefully inverted. After incubation for 2 h the samples were centrifuged for 2 min at 500 g. The supernatant was then discarded, and the pellet was resuspended in 5 mL 1x PBS (storage up to several weeks at 4 °C was possible). Before the blood samples were spotted onto slides, they were centrifuged for 2 min at 500 g. The supernatant was discarded; the pellet was resuspended in 10 mL 0.5 M sodium cacodylate buffer, and centrifuged for 5 min at 500 g. Then the supernatant was discarded, and the pellet was resuspended in 1 mL 0.05 M sodium cacodylate buffer. Afterwards, a 100 µL cell suspension were settled on carbon coated cover slips (Assistant) using a Cytospin 2 centrifuge (Shandon, Dako-Diagnostica) for 2 min at 1200 rpm. Samples were dehydrated in increasing concentrations of acetone (from 70 % to 100 %). Due to critical point drying (BAL-TEC CPD 030, Critical Point Dryer), acetone was replaced by CO_2. Finally, the slides were mounted on aluminium stubs, which were sputtered rotating at 45 ° with 4 nm of platinum/carbon using the BAL-TEC MED 020 coating system and analysed on a Zeiss Supra 50 VP scanning electron microscope.

IV.4 General methods for preparation of DNA

IV.4.1 Purification of genomic DNA

IV.4.1.1 BEL lysis

400 µL of blood was mixed with BEL buffer (1 M Tris (pH 7.4), 1 M $MgCl_2$, 500 µL Triton X-100, 1 M sucrose, ad 50 mL H_2O_{MQ}) at a 1:1 ratio and centrifuged at 10000 rpm (Eppendorf Mikrozentrifuge) for 1 min. The supernatant was discarded, and the pellet was resuspended in 400 µL BEL buffer and centrifuged again. The BEL buffer wash was repeated so long as the pellet remained colourless. Then, the pellet was resuspended in 400 µL 1x PBS.

IV.4.1.2 DNA purification

Genomic DNA was prepared by BEL lysis and adjacent purification using several methods. DNA was stored at -20 °C until use.

First, the phenol extraction method was used. The BEL-lysed pellet was resuspended in 400 µL 1x PBS and digested by Proteinase K: 45 µL 10 % SDS (Sigma-Aldrich), 22.5 µL TEN buffer (100 mM Tris, 10 mM EDTA, 1 M NaCl, pH 7.4), and 10 µL Proteinase K (50 U/mL, Roche) were added and incubated for 2 h at 56 °C. Afterwards, 400 µL phenol (Rotiphenol, Roth) were added, and the tube was inverted approx. 100x before incubation for 20 min on ice and centrifugation for 5 min at 10000 rpm and RT (Eppendorf Mikrozentrifuge). The supernatant was detached and phenol extraction was repeated. Again, the supernatant was detached and mixed with 400 µL IAC (Sigma-Aldrich). The tube was then inverted approx. 30x and centrifuged for 5 min at 10000 rpm and RT. The supernatant was removed and IAC extraction was repeated. Finally, the DNA was precipitated using EtOH. To do this, the supernatant was mixed with 40 µL NaAc and 800 µL $EtOH_{100\,\%}$ (Fluka), incubated for 30 min at -70 °C (alternatively for 24 h at -20 °C), and centrifuged for 30 min at 15000 rpm and 4 °C (Heraeus Biofuge Stratos, Fisher Scientific). The supernatant was discarded. Then, the pellet was washed in 400 µL $EtOH_{70\,\%}$, centrifuged for 30 min at 15000 rpm (Heraeus Biofuge Stratos) and 4 °C, and the supernatant was discarded. The pellet was air-dried and resuspended in 20 µL H_2O_{MQ}.

Second, three commercial kits were used to isolate DNA according to manufacturer's instructions: Sigma Gene Elute Bacterial Genomic DNA Kit (Sigma-Aldrich), the Qiagen DNeasy blood & tissue kit (Qiagen), and the MagNAPure System (Roche).

Third, the Looxster® Kit (SIRS-Lab) was used to selectively enrich bacterial DNA rather than eukaryotic DNA.

IV.4.2 Purification of plasmid DNA

Isolation of plasmid DNA was carried out with the GeneElute™ Plasmid Miniprep Kit (Sigma-Aldrich) according to the manufacturer's protocol.

IV.4.3 Analysis of purity and concentration of DNA

DNA concentration measurements were performed by detecting absorption at 260 nm (BioPhotometer, Vaudaux Eppendorf). Additionally, 5 µL of DNA was applied on an 1 % agarose gel to check purity.

IV.5 Agarose gel electrophoresis

Gel electrophoresis was used to verify size, purity, and concentration of nucleic acids. Standard ladders were used to evaluate fragment size and to estimate concentration of samples (100 bp DNA ladder, 1 kb DNA ladder; New England Biolabs).

The appropriate amount of agarose (AGAROSE Standard Molecular Biology Grade, Eurobio) was dissolved in 1x TAE buffer (Sigma-Aldrich) and boiled. Per 100 µL TAE-agarose-solution, 3 µL EtBr (Merck) was added, and the gel was cast in a gel chamber. Additionally, 3 µL of EtBr per 100 mL running buffer (1x TAE) were added in the gel chamber. Samples were mixed 1:10 with 10x loading dye. For DNA fragments with size greater than 500 bp, a bromphenol blue buffer (0.25 % bromphenol blue, 30 % glycerol; running dye front: 500 bp) was used, and a xylene-cylene blue buffer (0.25 % xylene-cylene, 30 % glycerol; running dye front: 1200 bp) was used for smaller fragments. Electrophoresis was performed with a constant current of about 100-120 mA for about 20-45 min. The agarose gel was photographed on an UV transilluminator.

IV.6 In vitro DNA amplification by polymerase chain reaction

IV.6.1 Primer

The primers used in this study were constructed using the following rules and were checked by OligoAnalyzer 3.1 (Integrated DNA Technologies, Coralville, USA; http://eu.idtdna.com/analyzer/applications/oligoanalyzer/default.aspx).

- Keep GC%-content around 50 %
- Set length between 17-28 nucleotides and avoid distinct secondary structures
- Put a 'C' at the 3'-end ideally
- Ensure forward and reverse primer have approx. dissociation temperatures
- Avoid more than three identical bases in a row

All oligonucleotides used in this study are listed in Table IV-4 (p. 39) and were synthesised and purified by Eurofin MWG Operon (Ebersberg, GER).

IV.6.2 Conventional PCR

All PCR reactions were performed in a mastercycler gradient (Eppendorf) or in a thermal cycler (Type 2400, Applied Biosystems) using the Taq DNA polymerase (Roche), and all reagents were kept on ice. 2 µL of template DNA was mixed with 23 µL of master mix no. 1 and 25 µL of master mix no. 2 (Tab. IV-3).

Table IV-3 Standard 50 µL protocol using the Taq DNA polymerase (Roche)

Component	Concentration	[µL]	No.	Temperature	Time
	master mix no. 1				
H_2O_{MQ}		21.6	1x	95 °C	2 min
dNTPs	10 mM	0.4		94 °C	30 s
Primer for	100 pmol/µL	0.5	35-40x	x °C	45 s
Primer rev	100 pmol/µL	0.5		72 °C	y
			1x	72 °c	10 min
	master mix no. 2				
H_2O_{MQ}		19.75			
Buffer	10x	5			
Taq		0.25			

No. = number of cycles, x = primer dependent annealing temperature, y = elongation time dependent on the fragment length

Table IV-4 Oligonucleotide primers and probes used in this study

Name	Application	5'-sequence-3'	T_M [°C]	GC [%]	Reference
27F	F, 1	CAGAGTTTGATCCTGGCTCAG	59.8	52.4	L. HOELZLE (IVB)[a]
1492R	R, 1	TACGGYTACCTTGTTACGACTT	57.5	43.2	L. HOELZLE (IVB)[a]
h1f	F, 1	CAGCCRCAATGGGATTGA	54.8	52.8	L. HOELZLE (IVB)[a]
h2f	F, 1	GGCCCATATTCCTRCGGGAAG	62.8	59.5	L. HOELZLE (IVB)[a]
h1r	R, 1	TAGTTTGACGGGCGGTGTGTA	59.8	52.4	L. HOELZLE (IVB)[a]
h2r	R, 1	ACRGGATTACTAGTGATTCCA	54.9	40.5	L. HOELZLE (IVB)[a]
Mh_F4	F, 1	AGCAGCAGTAGGGAATCTTC	57.3	50.0	314
Mh_R3	R, 1	TTCAAGGAGGCGAATTGCAG	57.3	50	314
80F1	F, 2	GAGGAAAGTCCRYGCTWGCAC	61.8	57.1	W. LUDWIG (TUM)[a]
290R1	R, 2	TCCCYTACCRAAATTTRGGTTTCT	58.4	39.6	W. LUDWIG (TUM)[a]
SYBR_For	F, 3	AGCAATRCCATGTGAACGATGAA	58.0	41.3	366
SYBR_Rev1	R, 3	TGGCACATAGTTTGCTGTCACTT	58.9	43.5	366
SYBR_Rev2	R, 3	GCTGGCACATAGTTAGCTGTCACT	62.7	50	366
MycHorse_F1	F	GCAAGCGCAGGCGGATGTG	63.1	68.4	This study
MycHorse_F2	F	GCGCAGGCGGATGTGTAAG	61.0	63.2	This study
MycHorse_F3	F, 3	CAGGCGGATGTGTAAGTTC	56.7	52.6	This study; 63
MycHorse_R1	R	GCCTAAGCGTCAATTATGGCC	59.8	52.4	This study
MycHorse_R2	R	CCTAAGCGTCAATTATGGCC	57.3	50.0	This study
MycHorse_R3	R, 3	CGCCTCCGGTGTTCTTAAAC	59.4	55.0	This study; 63
M13uni	F, 4	GTAAAACGACGGCCAG	50.7	56.3	TOPO TA Cloning Kit[b]
M13rev	R, 4	CAGGAAACAGCTATGAC	47.0	47.1	TOPO TA Cloning Kit[b]
EUB	5	GCTGCCTCCCGTAGGAGT	55.0	66.7	7
NON-EUB	5	ACTCCTACGGGAGGCAGC	55.0	66.7	352

F = forward primer, R = reverse primer, 1 = 16S rRNA PCR, 2 = *rnpB* PCR; 3 = SYBR green I real-time PCR, 4 = colony screen of transformed *E. coli* cells, 5 = CARD-FISH probes labelled with HRP; IUPAC codes: Y = C or T, R = A or G, W = A or T; IVB = Institute of Veterinary bacteriology, University of Zurich; TUM = Technische Universität München; [a] = personal communication; [b] = Invitrogen

IV.6.3 Gradient PCR

In order to evaluate the optimal annealing temperature of newly designed oligonucleotide primers, gradient PCR was performed in a mastercycler gradient (Eppendorf) with a gradient in the annealing temperatures of about 10 °C using a 50 µL standard protocol (Tab. IV-3, p. 38).

IV.6.4 Purification of PCR fragments

PCR products were purified from remaining nucleotides, polymerases, primers, and salts using the QIAquick PCR Purification Kit (Qiagen). The manufacturer's protocol was followed.

IV.6.5 Quality control of PCR products

All PCR products were checked qualitatively and quantitatively by agarose gel electrophoresis.

IV.6.6 Real-time PCR

IV.6.6.1 Universal HM screening SYBR green I real-time PCR assay

The universal HM screening SYBR green I real-time PCR assay (366) was performed to check negative control samples for HM infection.

IV.6.6.2 SYBR green I real-time PCR assay specific for the equine HM isolate

During this study, a SYBR green I real-time PCR assay specific to the novel equine HM isolate was developed and published ((63); pp. 140-164).

IV.7 PCR fragment cloning

Amplified DNA fragments were cloned with the TOPO TA Cloning Kit (Invitrogen). One Shot® chemical competent *E. coli* Top10 cells were used in conjunction with the pCR® 2.1-TOPO® cloning vector. To insert the fragments into the cloning vector, 4 µL purified PCR product, 1 µL vector, and 1 µL salt solution were mixed and incubated for 15 min at RT. Then, the mixture was added to frozen competent cells and incubated for 20 min on ice. Cells were heat-shocked at 42 °C for 30 s and incubated in 250 µL prewarmed S.O.C. medium for 1 h at 37 °C while shaking. 70 µL and 200 µL of transformed bacteria were plated on LBZ agar plates each (Ch. IV.2, p. 34) and incubated for 16 h at 37 °C. Clones were picked, incubated in 50 µL LB medium (Tab. IV-2, p. 33; supplemented with ampicillin at a final concentration of 100 µg/mL) for 1 h at 37 °C. A

colony screen PCR using 2 µL bacterial culture as template, M13 primers, and a standard protocol was performed (Ch. IV.6.2., p. 40).

IV.8 Sequencing of DNA

Sequencing was performed by Eurofin MWG Operon (Ebersberg, GER).

IV.9 CARD FISH

PERNTHALER *et al.* and SCHÖNHUBER *et al.*'s protocol (242, 278) were used with slight modifications.

IV.9.1 Preparation of samples and slides

50 µL EDTA-anti-coagulated blood was incubated in 2.5 mL PBS-GB (1x PBS, 10 mM glucose (Sigma-Aldrich), 0.1 % BSA (Sigma-Aldrich)) for 15 min at 37 °C. Afterwards, 1 mL blood-PBS-GB was incubated in 1 mL PFA-GA (1x PBS, 8 % PFA (Sigma-Aldrich), 0.02 % GA (Sigma-Aldrich)) for 24 h at 4 °C, centrifuged for 2 min at 500 g, and resuspended in 1x PBS.

Ten-well slides (Erie Scientific Company, Diagnostic Microscope Slide) were coated with 20 µL poly-L-lysine (25 µg/mL; Roth) per well for 1 h at RT and rinsed with H_2O_{MQ}. 10 µL of fixed blood were applied to each well and air-dried at 37 °C. Samples were dehydrated using an EtOH series (50 %, 80 %, and 100 %): 20 µL per well, each for 3 min at RT. Finally, each well was incubated in 20 µL $EtOH_{100\,\%}$ (Fluka), and the slides were air-dried at 37 °C.

IV.9.2 Permeabilisation and enzymatic pre-treatment

Each well was incubated in 50 µL permeabilisation buffer (100 mM Tris/HCl (pH 7.5), 50 mM EDTA (pH 8.0), 1 mg lysozyme (Roche), ad 100 mL H_2O_{MQ}) for 30 min at 37 °C, rinsed with H_2O_{MQ}, and the remaining liquid was removed by filter paper before air-drying the slides at 37 °C. Endogenous peroxidases were inactivated by incubation in 20 µL 0.01 M HCl per well for 10 min at RT, slides were washed in H_2O_{MQ} and $EtOH_{96\,\%}$, air-dried at 37 °C, and incubated in 20 µL 3 % H_2O_2 per well for 10 min at RT. Finally, the slides were washed in H_2O_{MQ} and $EtOH_{96\,\%}$ and air-dried at 37 °C.

IV.9.3 Hybridisation and washing

Hybridisation buffer (0.9 M NaCl, 20 mM Tris/HCl (pH 8.0), 35 % formamide (Roth), 10 % dextran sulphate (Sigma-Aldrich), 1 % blocking buffer (Roche), 0.02 % SDS (Sigma-Aldrich)) was prepared freshly each time. SDS was added last to avoid precipitation with the concentrated NaCl. The buffer was heated to 40-60 °C and shaken until dextran sulphate was completely dissolved. The probe solution (50 ng/µL) was mixed with hybridisation buffer at a 1:100 ratio. 50 µL hybridisation buffer (cooled to 35 °C) were applied per well. Then, the samples were incubated for 20 min at 35 °C, and the hybridisation buffer was removed with filter paper. 50 µL fresh hybridisation buffer were added and incubated for 2 h at 35 °C. Incubation was performed in a humidity chamber inlayed with blotting paper soaked with the remaining hybridisation buffer without the probe.

The hybridisation buffer was removed, and the slides were quickly and carefully rinsed with washing buffer (33.6 mM NaCl, 20 mM Tris/HCl (pH 8.0), 5 mM EDTA (pH 8.0), 0.01 % SDS). The wells were covered with washing buffer, and then the slides were transferred to a humidity chamber inlayed with blotting paper soaked with washing buffer and incubated for 25 min at 37 °C. The washing buffer was removed with filter paper, the slides were incubated in PBS-T (1x PBS supplemented with 0.05 % Triton X-100 (Sigma-Aldrich)) for 15 min at RT, carefully rinsed with H_2O_{MQ}, and the remaining liquid was removed with filter paper.

IV.9.4 Tyramide signal amplification

After hybridisation, the slides were immediately transferred to the substrate mix (1:10 tyramide-Cy5:amplification buffer (TSA™ Fluorescence system; NEN® Life Science Products) in the dark. 10 µL of amplification buffer were added per well before incubation for 20 min at RT. Slides were briefly placed onto blotting paper, washed in PBS-T for 10 min, in excess H_2O_{MQ} and then in $EtOH_{100\%}$ for 1 min at RT, and air-dried.

IV.9.5 Counterstaining

IV.9.5.1 DAPI

Cells were stained with 20 µL DAPI (1 µL stock solution (100 µg/mL), ad 20 mL 1x PBS; Sigma-Aldrich) per well for 5 min at RT, washed three times in 1x PBS, once in $EtOH_{80\,\%}$, and air-dried.

IV.9.5.2 Glycophorin A stain

Due to cross-reactivity of porcine and equine membrane components, RBC surfaces were stained using a purified mouse anti-pig CD235a (glycophorin A) monoclonal antibody (1:100, PharMingen, BD Biosciences) for 45 min at 37 °C, followed by FITC-conjugated goat anti-mouse IgG (1:100, Sigma) for 45 min at 37 °C. After, slides were washed three times in 1x PBS and air-dried.

IV.9.6 Microscopy and image analysis

Cover slips (22x50 cm, Menzel) were mounted by use of 1 drop glyzergel + DABCO (Invitrogen) per well. Samples were analysed using a Leica SP2 confocal microscope.

IV.10 Statistical analysis

Data were compiled and analysed using Excel (Microsoft) and R Foundation for Statistical Computing (R version 2.11.1). Categorical values (gender, anaemia, clinical status, pregnancy, type of housing) were analysed by Fisher's exact test and continuous variables (age, haematological parameter) using the Mann-Whitney *U*-test. Correlation of haematocrit and blood loads was assessed by the Spearman rank correlation coefficient (r_s). Differences were considered statistically significant if $P \leq 0.05$.

IV.11 Phylogenetic analysis

Phylogenetic analyses were carried out applying the ARB software package (187). Sequences were aligned using the ClustalW tool, and the alignment was refined manually. Reference sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/nucleotide/). For 16S rRNA genes, seed alignment with corresponding secondary structure annotation was provided by DR. WOLFGANG LUDWIG (TU München). Trees were calculated using a neighbour joining and a maximum likelihood approach (RAxML) in combination with similarity filters of 0 %, 25 %, and 50 % (185), respectively. Trees were re-sampled 1000 times. Only sequences longer than 1200 bp were taken in consideration for tree calculation. Shorter sequences were added after initial tree calculation using a maximum parsimony approach.

V. RESULTS

V. RESULTS

V.1 Haemotrophic mycoplasmas in horses

V.1.1 Clinical signs

Horses infected with HM displayed unspecific clinical signs including reduced general health, apathy, reduced performance, exercise intolerance, asthenia, and weight loss. In young horses, developmental retardation may occur. Changes in vital parameters (pulse, respiration, temperature) were not usually detected. In 27 of 156 samples, for which a complete blood count was available (17.3 %), a normocytic, normochromic anaemia was found, and a microcytic, hypochromic anaemia was observed in twelve cases (7.7 %).

V.1.2 Haematological analysis

V.1.2.1 Complete blood counts

Correlation between HM infection and anaemia was examined through analysis of complete blood counts from 156 samples collected during Autumn and Winter 2009 and 2010 (no. 001-007, 010-012, 014, 015, 020-026, 029-031, 036, 048-157; Tab. VIII-1, pp. 165-175). Results of haematological values are summarised in Table V-1 (pp. 46-50) and Figure V-1 (pp. 51-54). Detailed presentation of blood counts can be found in Table VIII-3 (pp. 180-187).

V.1.2.2 Comparison of blood counts of HM infected and non-infected horses

Haematological parameters for PCR positive and PCR negative (SYBR green I real-time PCR specific for equine HM; Ch. IV.6.6.2, p. 40; (63); pp. 140-164) horses are compared in Table V-1 (pp. 46-50) and Figure V-1 (pp. 51-54). To establish reference ranges, the data obtained by LATIMER & RAKICH (178) was used. In PCR positive horses, RBCC and MCHC were significantly lowered ($P = 0.00042$ and $P = 0.03434$, respectively). Changes in other blood parameters were not as significant, although the data exhibited slight decreases in haemoglobin concentration ($P = 0.08337$), absolute number of eosinophils ($P = 0.06017$), and relative number of eosinophils ($P = 0.09826$) in infected animals. Decreases in RBCC and haemoglobin concentration may indicate haemolytic anaemia.

Table V-1 Summary of results of complete blood counts

Parameter	Ref. range	Mean	Range	Median	1st Qu	3rd Qu
		All samples (n = 118)				
Leucocytes	6.0-12.0 G/L	9.64	3.60-14.30	9.80	7.73	11.18
Erythrocytes	6.0-12.0 T/L	8.79	6.54-13.38	8.84	8.28	9.28
Haemoglobin	100-180 g/L	128.0	94.0-212.0	128.0	120.0	137.0
Haematocrit	0.32-0.48 L/L	0.34	0.26-0.52	0.34	0.32	0.37
MCV	34-58 fL	39.41	30.90-50.80		35.42	43.18
MCH	13-19 pg	14.63	11.70-17.90	14.50	13.30	15.80
MCHC	31-37 g/dL	37.25	32.60-41.00	37.30	36.40	38.08
Thrombocytes	100-600 G/L	171.4	83.0-320.0	164.5	131.0	198.2
Neutrophils	30-75 %	46.25	20.00-66.00	48.00	40.25	53.00
Neutrophils abs.	3000-6000 /µL	4346	1332-8400	4246	3686	4972
Lymphocytes	25-60 %	46.05	23.00-74.00	44.00	37.00	53.50
Lymphocytes abs.	1500-5000 /µL	4574	1736-9870	4313	3000	5548
Monocytes	1-8 %	4.27	0-8.00	4.00	4.00	5.00
Monocytes abs.	0-100 /µL	401.4	0-1120.0	376.0	288.5	494.2
Eosinophils	1-10 %	2.77	0-7.0	3.0	2.0	4.0
Eosinophils abs.	0-800 /µL	255.0	0-756.0	240.5	145.0	364.8
Basophils	0-3 %	0.65	0-4.00	1.00	0.00	1.00
Basophils abs.	0-300 /µL	60.65	0-564	63	0	96.75

Table V-1 continued

Parameter	Ref. range	Mean	Range	Median	1st Qu	3rd Qu	Mean	Range	Median	1st Qu	3rd Qu	*P**
		All samples of horses over one year (n = 87)					All samples of horses under one year (n = 31)					
Leucocytes	6.0-12.0 G/L	9.18	3.60-14.10	9.20	7.45	10.80	10.91	7.50-14.30	10.90	9.70	11.95	$P = 0.00014^*$
Erythrocytes	6.0-12.0 T/L	8.71	6.54-13.38	8.80	8.17	9.21	9.00	7.09-10.65	9.03	8.36	9.55	$P = 0.03635^*$
Haemoglobin	100-180 g/L	132.7	112.0-212.0	132.0	124.0	140.0	114.8	94.0-131.0	114.0	109.5	121.5	$P < 0.00001^*$
Haematocrit	0.32-0.48 L/L	0.36	0.31-0.52	0.36	0.34	0.38	0.31	0.26-0.35	0.31	0.29	0.32	$P < 0.00001^*$
MCV	34-58 fL	41.35	33.40-50.80	41.00	37.15	44.40	33.98	30.90-38.30	33.60	32.70	34.85	$P < 0.00001^*$
MCH	13-19 pg	15.33	12.90-17.90	15.30	14.20	16.35	12.71	11.70-13.90	12.70	12.40	13.10	$P < 0.00001^*$
MCHC	31-37 g/dL	37.18	32.60-41.00	37.20	36.30	38.05	37.45	35.80-39.30	37.70	36.70	38.05	n. s.
Thrombocytes	100-600 G/L	158.7	83.0-272.0	153.0	128.5	186.5	207.1	103.0-320.0	201.0	166.0	247.0	$P = 0.00005^*$
Neutrophils	30-75 %	48.31	24.00-66.00	50	44	54	40.48	20-60	41	34	46	$P = 0.00004^*$
Neutrophils abs.	3000-6000 /µL	4321	1332-6825	4224	3777	4749	4416	2356-8400	4601	3354	5302	n. s.
Lymphocytes	25-60 %	43.47	23-74	42	36	48	53.29	32-74	52	46	60	$P < 0.00001^*$
Lymphocytes abs.	1500-5000 /µL	4122	1736-9870	3686	2743	4761	5842	3450-9408	5424	4669	6702	$P < 0.00001^*$
Monocytes	1-8 %	4.25	0-7	4	4	5	4.32	0-8	4	3	6	n. s.
Monocytes abs.	0-100 /µL	378.8	0-810	360	289	444	465	0-1120	456	312	572	$P = 0.0084^*$
Eosinophils	1-10 %	3.17	0-7	3	2	4	1.65	0-5	2	0	3	$P = 0.00002^*$
Eosinophils abs.	0-800 /µL	287	0-756	264	179	387.5	165.2	0-535	172	0	270.5	$P = 0.00049^*$
Basophils	0-3 %	0.79	0-4	1	0	1	0.26	0-2	0	0	0	$P = 0.00003^*$
Basophils abs.	0-300 /µL	72.28	0-564	75	0	99.5	28.03	0-264	0	0	0	$P = 0.00014^*$

Table V-1 continued

Parameter	Ref. range	Mean	Range	Median	1st Qu	3rd Qu	Mean	Range	Median	1st Qu	3rd Qu	*P**
		All PCR positive horses (n = 31)					All PCR negative horses (n = 87)					
Leucocytes	6.0-12.0 G/L	9.41	5.30-13.80	9.2	7.45	11.1	9.72	3.6-14.3	9.8	7.95	11.25	n. s.
Erythrocytes	6.0-12.0 T/L	8.39	6.85-10.32	8.37	7.77	8.94	8.93	6.54-13.38	8.87	8.40	9.51	$P = 0.00042^{*}$
Haemoglobin	100-180 g/L	124.5	94.0-150.0	124.0	115.5	132.5	129.2	101-212	129	120	137.5	$P = 0.08337$
Haematocrit	0.32-0.48 L/L	0.34	0.26-0.41	0.34	0.31	0.36	0.35	0.28-0.52	0.35	0.32	0.37	n. s.
MCV	34-58 fL	40.15	32.70-49.60	38.8	36.2	43.9	39.15	30.90-50.80	38.1	35.1	42.85	n. s.
MCH	13-19 pg	14.82	12.40-17.90	14.7	13.6	16.3	14.57	11.70-17.90	14.5	13.15	15.8	n. s.
MCHC	31-37 g/dL	36.96	35.00-39.00	37.00	36.15	37.80	37.35	32.60-41.00	37.5	36.6	38.15	$P = 0.03434^{*}$
Thrombocytes	100-600 G/L	172.5	107.0-286.0	171	134.5	195.5	171	83.0-320.0	162	132	200	n. s.
Neutrophils	30-75 %	46.09	20.00-66.00	47.00	39.5	54.5	46.32	24-65	49	40.5	53	n. s.
Neutrophils abs.	3000-6000 /µL	4189	2536-6666	4218	3496	4652	4402	1332-8400	4272	3754	5004	n. s.
Lymphocytes	25-60 %	46.45	23.00-74.00	44	36.5	52	45.91	28.0-74	43	37.5	53	n. s.
Lymphocytes abs.	1500-5000 /µL	4546	1792-9408	4171	2768	5797	4584	1736-9870	4346	3058	5528	n. s.
Monocytes	1-8 %	4.42	0-6	5	3.5	5.5	4.22	0-8	4	4	5	n. s.
Monocytes abs.	0-100 /µL	403.8	159-732	428	304	472.5	400.6	0-1120	372	289	497.5	n. s.
Eosinophils	1-10 %	2.42	0-6	3	1	3	2.90	0-7	3	2	4	$P = 0.09826$
Eosinophils abs.	0-800 /µL	218.7	0-756	222	73.5	300	268	0-742	258	166	384	$P = 0.06017$
Basophils	0-3 %	0.65	0-2	1	0	1	0.66	0-4	1	0	1	n. s.
Basophils abs.	0-300 /µL	58.1	0-254	53	0	94.5	61.56	0-564	66	0	96.5	n. s.

Table V-1 continued

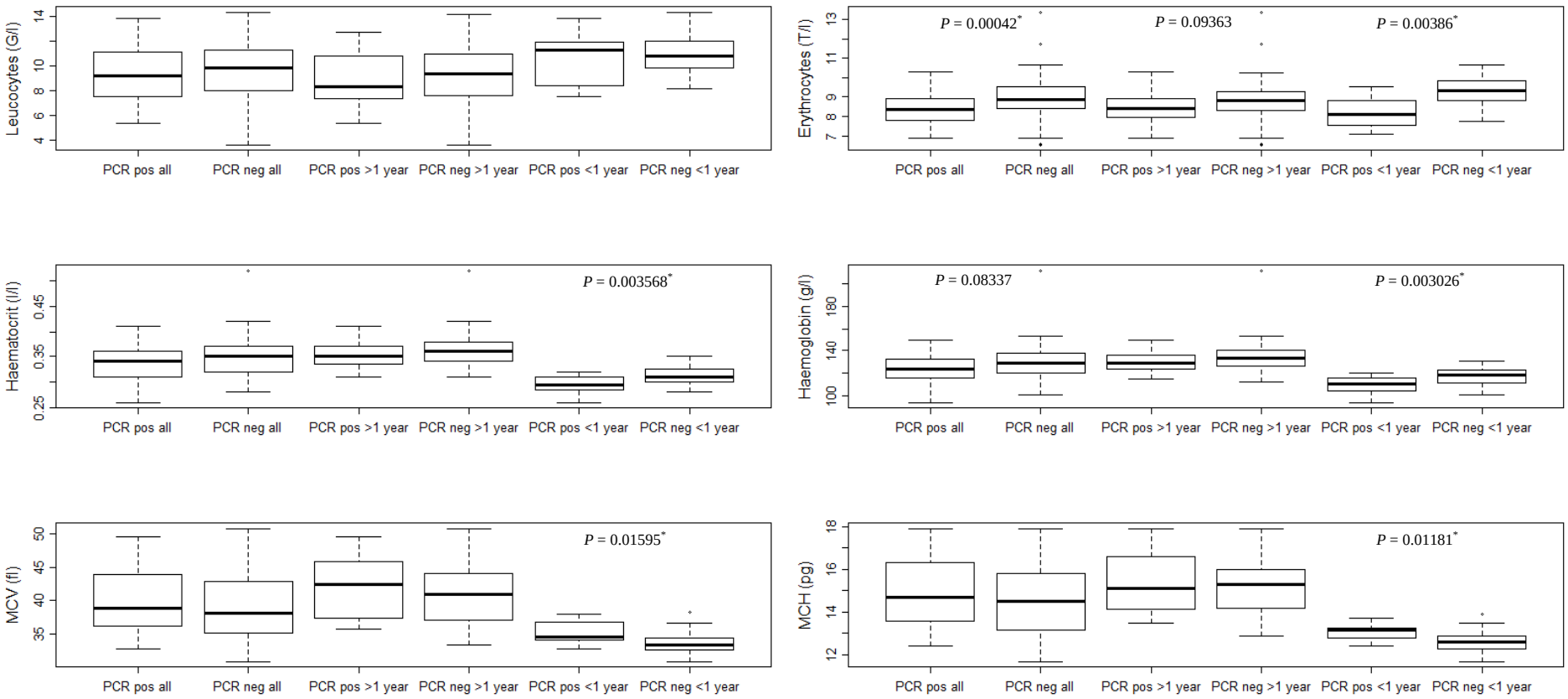
Parameter	Ref. range	Mean	Range	Median	1st Qu	3rd Qu	Mean	Range	Median	1st Qu	3rd Qu	*P**
		PCR positive horses over one year (n = 23)					PCR negative horses over one year (n = 64)					
Leucocytes	6.0-12.0 G/L	9.61	5.33-12.70	8.30	7.35	10.75	9.24	3.6-14.1	9.3	7.5	10.9	n. s.
Erythrocytes	6.0-12.0 T/L	8.46	6.85-10.32	8.42	7.95	8.94	8.8	6.54-13.38	8.81	8.29	9.25	P = 0.09363
Haemoglobin	100-180 g/L	129.8	115.0-150.0	129.0	124	135.5	133.7	111-212	133	126	140	n. s.
Haematocrit	0.32-0.48 L/L	0.35	0.31-0.41	0.35	0.34	0.37	0.36	0.30-0.52	0.36	0.34	0.38	n. s.
MCV	34-58 fL	41.88	35.70-49.60	42.40	37.35	45.85	41.16	33.40-50.8	40.9	37.17	43.95	n. s.
MCH	13-19 pg	15743	13.50-17.90	15.10	14.15	16.6	15.29	12.9-17.9	15.3	14.2	15.95	n. s.
MCHC	31-37 g/dL	36.9	35.00-39.00	37.00	36.15	37.8	37.28	32.6-41	37.3	36.4	38.2	P = 0.06836
Thrombocytes	100-600 G/L	165.1	107.0-272.0	159	129	192.0	156.4	83-242	151	128	180.5	n. s.
Neutrophils	30-75 %	48.91	26.00-66.00	48.00	44.5	56.5	48.09	24-65	50.5	44	54	n. s.
Neutrophils abs.	3000-6000 /µL	4276	2773-6666	4232	3694	4652	4337	1332-6825	4218	3778	4780	n. s.
Lymphocytes	25-60 %	42.96	23.00-70.00	44.00	34.50	47.5	43.66	28-74	41	37	48	n. s.
Lymphocytes abs.	1500-5000 /µL	4008	1792-7560	3652	2479	4818	4163	1736-9870	3691	2889	4673	n. s.
Monocytes	1-8 %	4.44	2-6	5	3.5	5	4.19	0-7	4	4	5	n. s.
Monocytes abs.	0-100 /µL	392	159-714	415	304	491.5	374.1	0-810	353	289.5	431.5	n. s.
Eosinophils	1-10 %	2.87	0-6	3.0	2	3.5	3.28	0-7	3	2	4	n. s.
Eosinophils abs.	0-800 /µL	262.3	0-756	238	1485	322.5	295.9	0-742	298.5	184.5	396.2	n. s.
Basophils	0-3 %	0.83	0-2	1	0	1	0.78	0-4	1	0	1	n. s.
Basophils abs.	0-300 /µL	75.04	0-254	74	0	107.50	71.28	0-564	75.5	0	98.25	n. s.

Table V-1 continued

Parameter	Ref. range	Mean	Range	Median	1st Qu	3rd Qu	Mean	Range	Median	1st Qu	3rd Qu	*P**
		PCR positive horses under one year (n = 8)					PCR negative under one year (n = 23)					
Leucocytes	6.0-12.0 G/L	10.54	7.5-13.8	11.25	8.8	11.68	11.03	8.1-14.3	10.8	9.8	12	n. s.
Erythrocytes	6.0-12.0 T/L	8.19	7.09-9.55	8.11	7.56	8.69	9.29	7.72-10.65	9.31	8.81	9.83	$P = 0.00386^{*}$
Haemoglobin	100-180 g/L	109.4	94-120	110.5	106.8	119.8	116.7	101-131	118	111	123	$P = 0.03026^{*}$
Haematocrit	0.32-0.48 L/L	0.30	0.26-0.32	0.30	0.29	0.31	0.31	0.28-0.35	0.31	0.30	0.33	$P = 0.03568^{*}$
MCV	34-58 fL	35.19	32.7-38.0	34.55	34.17	36.8	33.56	30.9-38.3	33.3	32.55	34.45	$P = 0.01595^{*}$
MCH	13-19 pg	13.00	12.4-13.7	13.15	12.9	13.22	12.59	11.7-13.9	12.6	12.3	12.9	$P = 0.01181^{*}$
MCHC	31-37 g/dL	37.14	35.8-38.5	37.25	36.15	38.02	37.56	35.7-39.3	37.7	37.05	38.05	n. s.
Thrombocytes	100-600 G/L	193.9	127-286	177	167.5	233.5	211.7	103.320	220	166.5	249.5	n. s.
Neutrophils	30-75 %	37.88	20-52	36.5	31.75	46.52	41.39	28-60	42	34.5	46	n. s.
Neutrophils abs.	3000-6000 /µL	3939	2356-5772	3726	3141	4737	4582	2610-8400	4635	3500	5302	n. s.
Lymphocytes	25-60 %	56.5	44-74	55	46	66	52.17	32-68	52	45.5	60	n. s.
Lymphocytes abs.	1500-5000 /µL	6096	3450-9408	5064	4590	7950	5754	3813-8908	5488	4770	6468	n. s.
Monocytes	1-8 %	4.38	2-6	4.5	3.5	6	4.30	0-8	4	3	6	n. s.
Monocytes abs.	0-100 /µL	438	230-732	453	402	457	516	0-1120	516	305	595	n. s.
Eosinophils	1-10 %	1.13	0-4	0	0	2.25	1.83	0-5	2	0	3	n. s.
Eosinophils abs.	0-800 /µL	93.25	0-368	0	0	169.5	190.2	0-535	196	0	286.5	$P = 0.0747$
Basophils	0-3 %	0.13	0-1	0	0	0	0.30	0-2	0	0	0	n. s.
Basophils abs.	0-300 /µL	9.38	0-75	0	0	0	34.52	0-264	0	0	0	n. s.

Reference (Ref.) ranges referred to LATIMER & RAKICH (178). MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, abs. = absolute. Mean, median and quartiles were calculated using R Foundation for Statistical Computing (R version 2.13.0). Blood counts of 156 horses were available. Fourteen horses' ages were unknown, so they were excluded from analyses. Also, 24 re-collected samples from 2009 were excluded. No samples exhibited an elevated number of segmented neutrophils or other cells. Samples were analysed by a one-sided Mann-Whitney *U*-Test. *P*-Values ≤ 0.1 are outlined. * significant, if $P \leq 0.05$; n.s. = not significant.

Figure V-1


Leucocytes (G/l)
4
6
8
10
14
PCR pos all
PCR neg all
PCR pos >1 year
PCR neg >1 year
PCR pos <1 year
PCR neg <1 year
Erythrocytes (T/l)
7
8
9
11
13
P = 0.00042*
P = 0.09363
P = 0.00386*
Haematocrit (l/l)
0.25
0.35
0.45
P = 0.003568*
Haemoglobin (g/l)
100
140
180
P = 0.08337
P = 0.003026*
MCV (fl)
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40
45
50
P = 0.01595*
MCH (pg)
12
14
16
18
P = 0.01181*

Figure V-1 continued

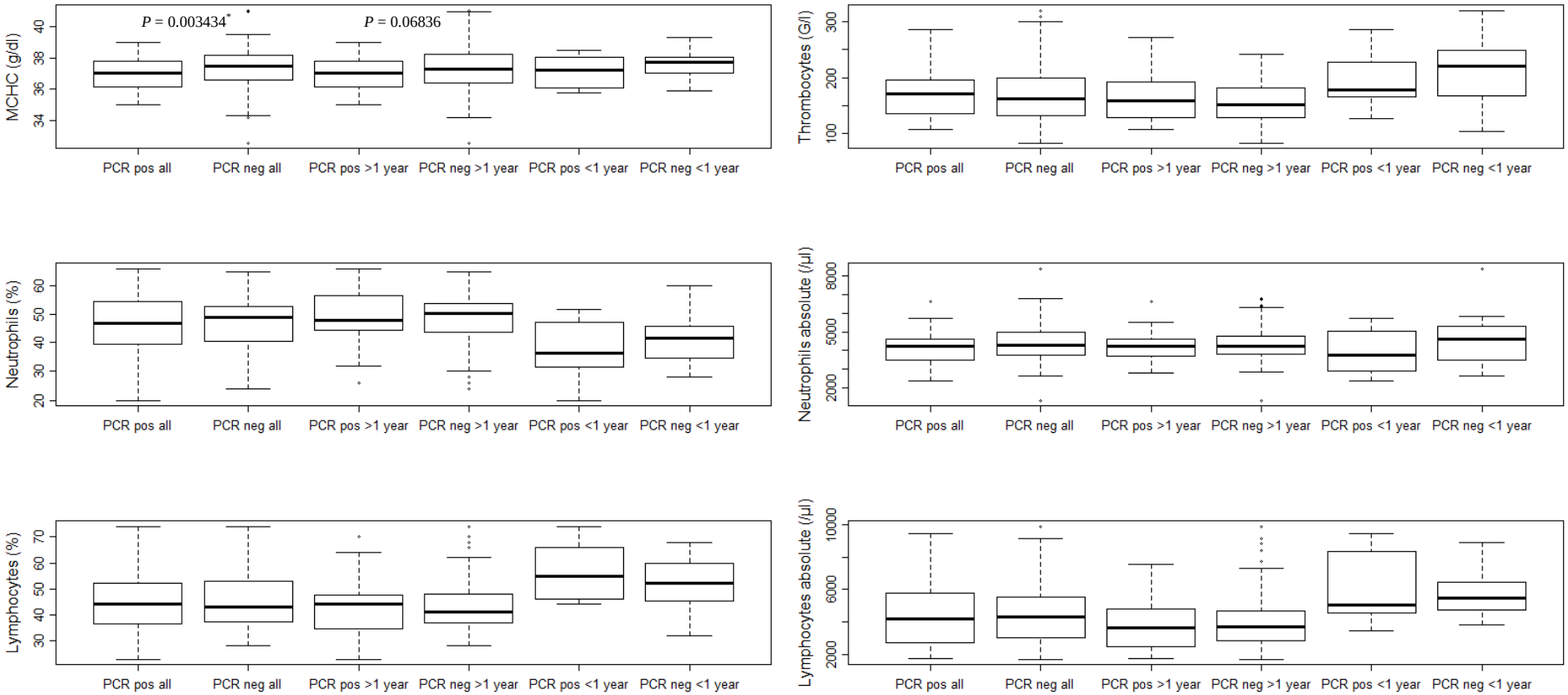

Figure V-1 continued

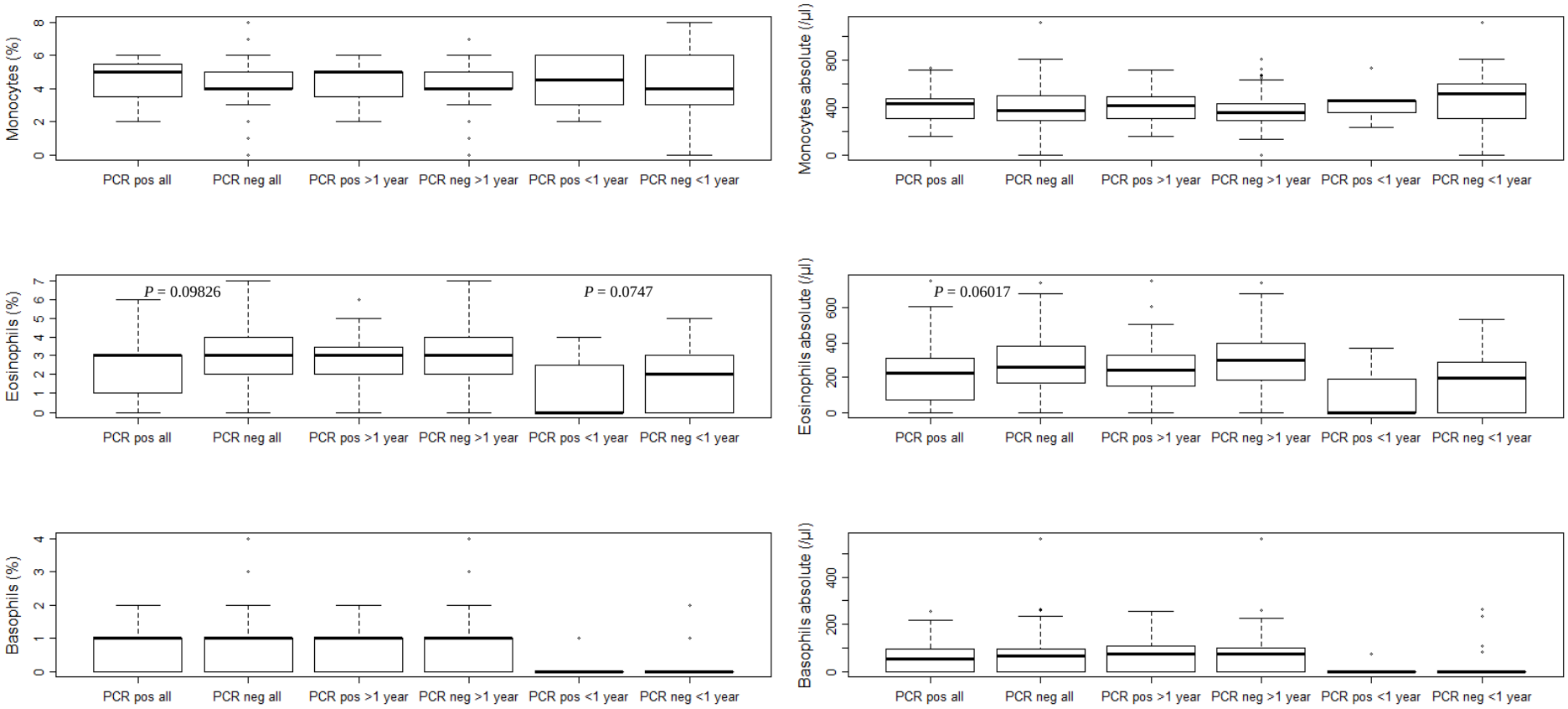

Figure V-1 Comparison of blood parameters for PCR positive (PCR pos) and PCR negative (PCR neg) horses. Blood counts of 156 horses were available. The ages of one PCR positive and thirteen PCR negative horses were unknown, and they were thus excluded from analyses. Twenty-four samples from 2009 that were re-collected one month later were also excluded. Results are shown for all horses (n = 118; PCR pos, n = 31; PCR neg, n = 87) and for horses grouped according to their age (PCR pos >1 year, n = 23; PCR neg >1 year, n = 64; PCR pos <1 year, n = 8; PCR neg <1 year, n = 23). No samples exhibited an elevated number of segmented neutrophils or other cells. Samples were analysed by a one-sided Mann-Whitney *U*-Test and boxplots were generated using R Foundation for Statistical Computing (R version 2.13.0). Boxes extend from the 25th to the 75th percentile, median is presented as horizontal line, and the bars extend to the minimum and maximum value. Dots represent outliers. *P*-Values ≤ 0.1 are outlined. * significant, if $P \leq 0.05$. Compare Table V-1 (pp. 46-50).

V.1.2.3 Differences of blood counts of young and adult horses

Haematological values for horses younger than one year and older than one year were compared by PCR status (Tab. V-1 & Fig. V-1, pp. 46-54). In PCR positive horses less than one year-old, the following blood parameter values were significantly lower; Decreases of RBCC ($P = 0.00386$), haemoglobin concentration ($P = 0.03026$), and haematocrit ($P = 0.03568$) indicated haemolytic anaemia. MCV and MCH were significantly elevated in PCR positive horses less than one year-old ($P = 0.01595$ and $P = 0.01181$, respectively). In contrast to these results, haematocrit was not significantly changed in PCR positive horses older than one year, and only slight decreases in RBCC and MCHC were found ($P = 0.09363$ and $P = 0.06836$, respectively).

Independent of PCR status, significant differences were found between PCR positive horses less than one year-old compared to horses older than one year (Tab. V-1, pp. 46-50; Fig. V-2, pp. 55-56).

Figure V-2

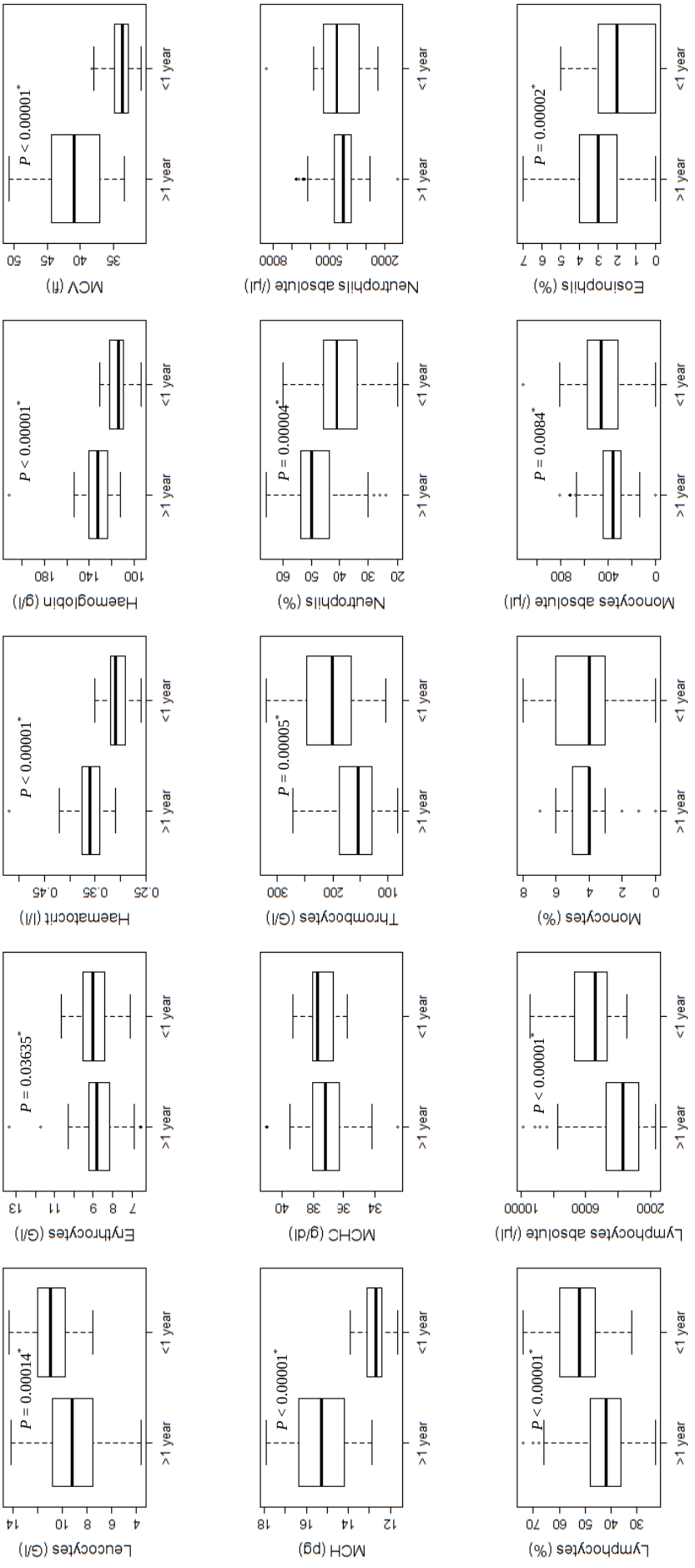
Leucocytes (G/l)
P = 0.00014*
>1 year
<1 year
MCH (pg)
P < 0.00001*
>1 year
<1 year
Lymphocytes absolute (/µl)
P < 0.00001*
>1 year
<1 year
Lymphocytes (%)
P < 0.00001*
>1 year
<1 year
Erythrocytes (G/l)
P = 0.03635*
>1 year
<1 year
MCHC (g/dl)
>1 year
<1 year
Thrombocytes (G/l)
P = 0.00005*
>1 year
<1 year
Monocytes absolute (/µl)
P = 0.0084*
>1 year
<1 year
Monocytes (%)
>1 year
<1 year
Haematocrit (l/l)
P < 0.00001*
>1 year
<1 year
Neutrophils (%)
P = 0.00004*
>1 year
<1 year
Neutrophils absolute (/µl)
>1 year
<1 year
Eosinophils (%)
P = 0.00002*
>1 year
<1 year
Haemoglobin (g/l)
P < 0.00001*
>1 year
<1 year
MCV (fl)
P < 0.00001*
>1 year
<1 year

Figure V-2 continued

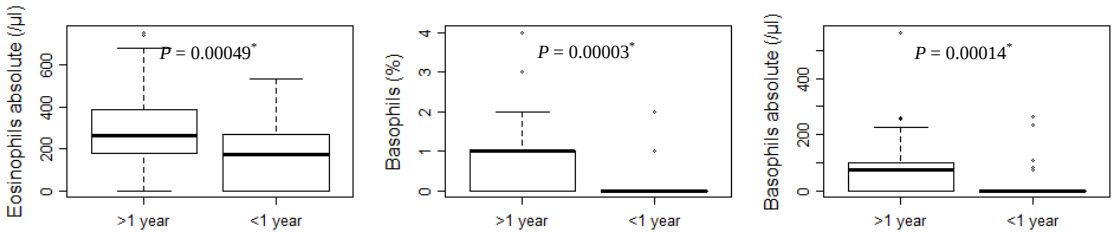

Figure V-2 Comparison of blood parameter values of horses older than one year (n = 87) and younger than one year (n = 31) independent of PCR status. Blood counts of 156 horses were available. Ages of one PCR positive and thirteen PCR negative horses were unknown, and they were therefore excluded from analyses. Twenty-four samples from 2009 that were re-collected one month later were also excluded. No samples showed an elevated number of segmented neutrophils or other cells. Samples were analysed using the one-sided Mann-Whitney *U*-Test, and boxplots were generated using R Foundation for Statistical Computing (R version 2.13.0). Boxes extend from the 25th to the 75th percentile, the median is presented as horizontal line, and the bars extend to the minimum and maximum value. Dots represent outliers. *P*-Values are outlined if significant ($P \leq 0.05$). Compare Table V-1 (pp. 46-50).

V.1.2.4 Cold agglutinins

In some PCR positive horse blood samples, agglutination induced by antibodies of the cold-type was seen. Agglutination repeatably dissolved upon incubation at 37 °C and was repeatedly re-induced by incubation at 4 °C. Cold agglutinins can be detected by Coombs' direct antiglobulin testing (353). Since the suspicion of cold agglutinins in horse blood was an incidental finding that occurred during sample preparation, the Coombs' testing was not performed immediately after sample collection but three months later, and, unfortunately, Coombs' testing failed.

Figure V-3 Cold Agglutinins. Left: negative horse sample, **middle:** strong agglutination, **right:** slight agglutination.

V.1.3 Microscopic Findings

V.1.3.1 Light microscopy

In peripheral blood smears stained with acridine orange and Giemsa, coccoid bacterial structures reminiscent of HM species which infect other animals were detected on the surface of equine RBCs (Fig. V-4). Also, typical rouleaux formation, and deformation and destruction of RBCs were observed. Sometimes, the HM lay in small grooves.

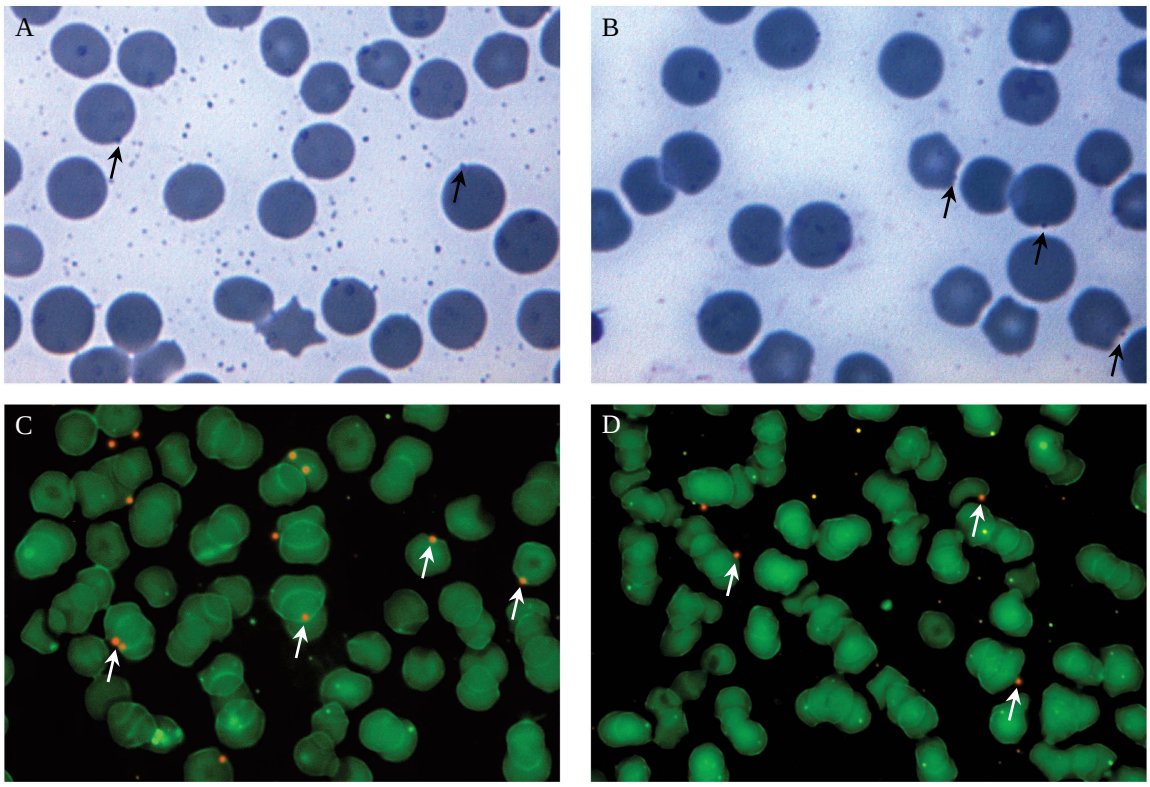

Figure V-4 Giemsa (A, B) and acridine orange (C, D) stained peripheral blood smears of horses infected with haemotrophic mycoplasmas. HMs are indicated by arrowheads. They are attached to RBCs and free in the plasma. Rouleaux formation and RBC deformation can be observed. Photographs were taken at 1000x magnification. Picture of Panel B was published slightly modified elsewhere (63).

Figure V-5

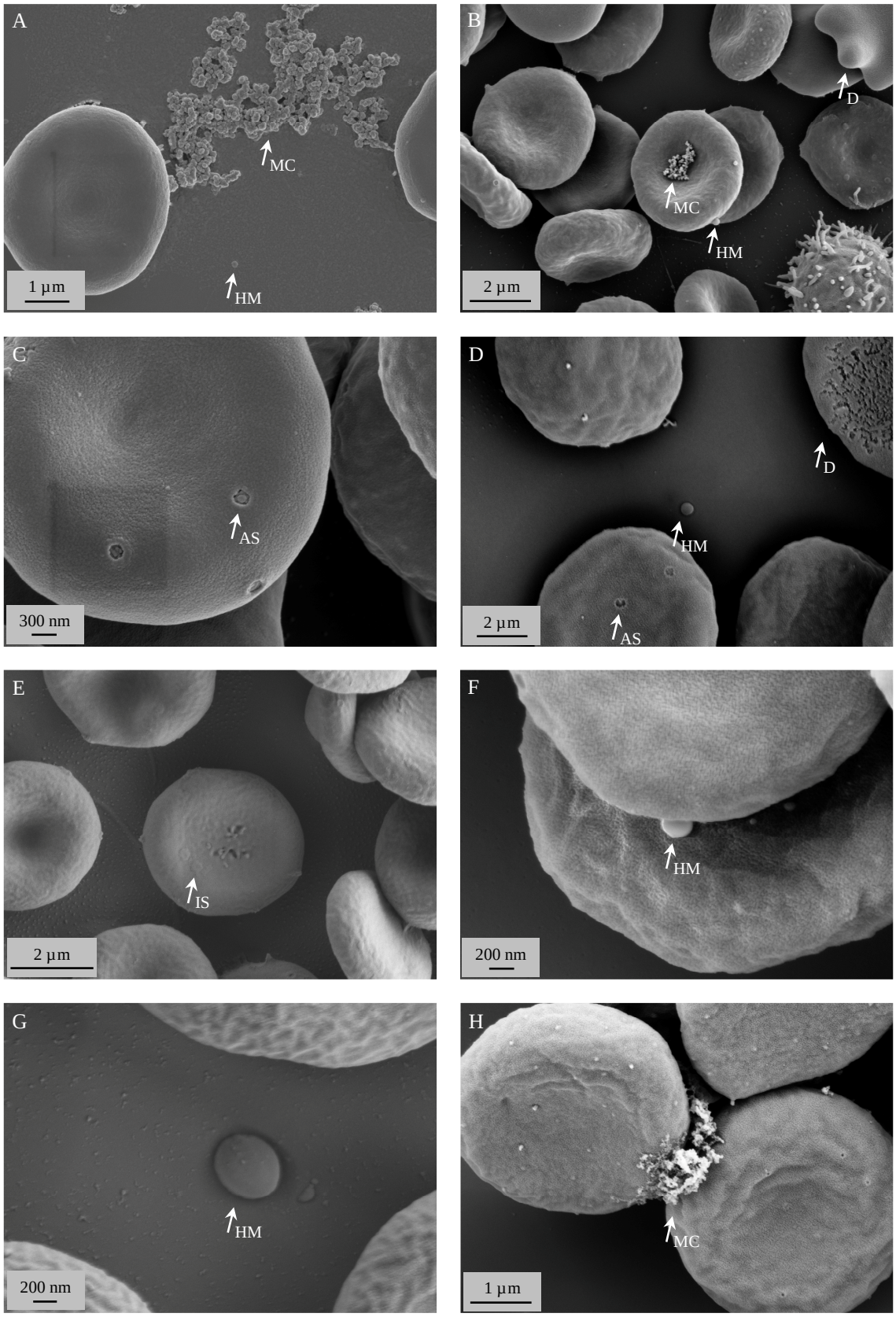
A
MC
HM
1 µm
B
D
MC
HM
2 µm
C
AS
300 nm
D
D
HM
AS
2 µm
E
IS
2 µm
F
HM
200 nm
G
HM
200 nm
H
MC
1 µm

Figure V-5 Scanning electron micrographs of equine red blood cells infected with haemotrophic mycoplasmas. Some RBCs are deformed and destructed (D). HMs were detected on the surface of and between RBCs (HM). Structures assumed to be attachment (AS) and invagination scars (IS) were observed as well as structures resembling microcolonies (MC).

V.1.3.2 Scanning electron microscopy

In scanning electron microscopy, destruction and deformation of RBCs was directly observed (Fig. V-5 B & D). Sometimes, HM-like particles attached to the surface were found (Fig. V-5 B & F). Unfortunately, HMs were frequently found detached from surface of RBCs and were instead detected between RBCs (Fig. V-5 A, D & G). Nevertheless, attachment scar-like structures were observed (Fig. V-5 C & D). Also, structures similar to *M. suis* invagination scars (106) were seen (Fig. V-5 E). In some samples, microcolony structures resembling natural *M. suis* infections in pigs were detected (DR. KATRIN GROEBEL, IVB, pers. comm.; Fig. V-5 A, B & H). In some samples, agglomeration of infected and/or deformed RBCs with WBCs was observed.

V.1.4 Cultivation

Cultivation was monitored by visual analysis of agar plates, subcultivation in blind passages, and 16S rRNA PCR. After failure to cultivate the first horse blood samples collected in February 2008, no further cultivation attempts were made.

V.1.5 DNA purification

Blood was lysed by the BEL lysis method and subsequently purified using standard methods. Phenol extraction exhibited highest DNA yield, but phenol contamination of samples can inhibit PCR and may interfere with SYBR green in real-time PCR. That is why, three commercial DNA purification methods (Sigma Gene Elute Bacterial Genomic DNA kit, Qiagen DNeasy blood & tissue kit, Roche MagNA Pure LC system) were tested. Efficiency of all three systems was found to be approximately equal, but the Sigma kit exhibited the best price-performance-ratio and was therefore chosen for DNA purification.

A selective enrichment of prokaryotic DNA relative to eukaryotic DNA was attempted using the Looxster® kit. However, the absolute DNA loss was high and therefore the DNA purification efficiency was poor, as HM blood loads were very low.

V.1.6 PCR & Sequencing

V.1.6.1 Conventional 16S PCR

64 of 103 samples (62.1 %) revealed a positive PCR result using a primer pair targeting universal 16S rRNA regions (27F/ 1492R) in a conventional PCR protocol. 57 of 105 (54.3 %) samples were HM positive as demonstrated by utilisation of a PCR primer pair specific for HM (hf1/ h1r). However, 16S rRNA sequencing (hf1/ h1r) was only successful in two cases. Comparison to databases using the BLAST tool revealed 97-98 % sequence identity with '*C*M haemobovis' and 93-94 % with *M. haemofelis* (Tab. V-7, p. 82) (62). Sequencing using the universal 16S rRNA primer pair failed to obtain full-length HM sequences. Instead, amplicons from contaminating bacteria were cloned, or no PCR products or clones were obtained. Detailed PCR results are presented in Table V-4 (pp. 69-73).

V.1.6.2 Design of equine HM specific PCR primer pairs

In two horses, 900 bp of partial 16S rRNA sequences were successfully amplified using primer pair hf1 and hr1 (62). These sequences were then used to design equine HM-specific PCR primer pairs. Oligonucleotides were designed by use of the probe design tool in the ARB software package (187), and primer specificity was tested *in silico* with the programme pDRAW (http://www.acaclone.com/ (334)). Possible primer pair sequences are outlined in Table IV-3 (p. 39) and their binding specificities in Table V-2 (p. 62). Primers were tested in a standard PCR protocol (Ch. IV.6.2, p. 38) at annealing temperatures of 55 °C and 58 °C. Optimal annealing temperatures were evaluated by gradient PCR (Ch. IV.6.3, p. 40).

Primer combinations amplifying HM from the 'haemominutum-group', '*C*M turicensis', *M. haemofelis*, or *M. haemocanis* were discarded. Only three primer combinations selectively amplified the 16S rRNA sequence of the novel equine HM isolate and '*C*M haemobovis' (F1/R3, F2/R3 and F3/R3). Since these two HM isolates are very closely related, it was not possible to find sufficient sequence differences for primer design exclusively to the equine HM isolate. These three combinations were then tested in a SYBR green I real-time PCR assay. The primer pair F3/R3 was subsequently chosen for design of the SYBR green I real-time PCR assay specific for the novel equine HM isolate, as it exhibited the highest sensitivity compared to the other two combinations, and it can be used with an annealing temperature of 58 °C, which enhanced specificity of PCR (Tab. V-2, p. 62).

Table V-2 Primer pairs tested for specificity for the equine HM isolate

	F1/R1	F1/R2	F1/R3	F2/R1	F2/R2	F2/R3	F3/R1	F3/R2	F3/R3
'haemominutum-group'									
M. ovis	-	-	-	-	-	-	-	-	-
M. suis	-	-	-	-	-	-	-	-	-
M. wenyonii	-	-	-	-	-	-	-	-	-
M. coccoides	-	-	-	-	-	-	+	+	-
'haemofelis-group'									
'*C*M turicensis'	-	-	-	+	+	-	+	+	-
M. haemomuris	-	-	-	-	-	-	-	-	-
M. haemofelis	+	+	-	+	+	-	+	+	-
M. haemocanis	+	+	-	+	+	-	+	+	-
'*C*M haemobovis'	+	+	+	+	+	+	+	+	+
Plasmid 30/7	+	+	+	+	+	+	+	+	+
PCR 55 °C	+	+	+	+	+	+	+	+	+
PCR 58 °C	-	+	-	-	+	-	+	+	+

V.1.6.3 SYBR green I real-time PCR assay

DIECKMANN *et al.*, 2011 ((63); pp. 140-164) describes the development of the SYBR green I real-time PCR assay. In this section, only supplementary material and results concerning samples not included in the aforementioned publication are presented.

Seventy of 211 horse samples (33.2 %) displayed positive SYBR green I real-time PCR signals. Table V-4 (pp. 69-73) summarises values of crossing points (CP), melting temperatures (T_M), and blood loads of all samples. The mean CP was 31.95 (range: 26.52-36.38), the mean T_M was 80.81 °C (range: 80.04-82.07 °C), and the mean blood load was 1.67 x 10^7 cells/mL blood (range: 1.70 x 10^2-3.69 x 10^8 cells/mL blood). Typical amplification and melting curves are shown in Figure V-6. High HM loads in blood were not associated with disease, anaemia, or abnormalities in blood count (Fig. V-7, pp. 64-66).

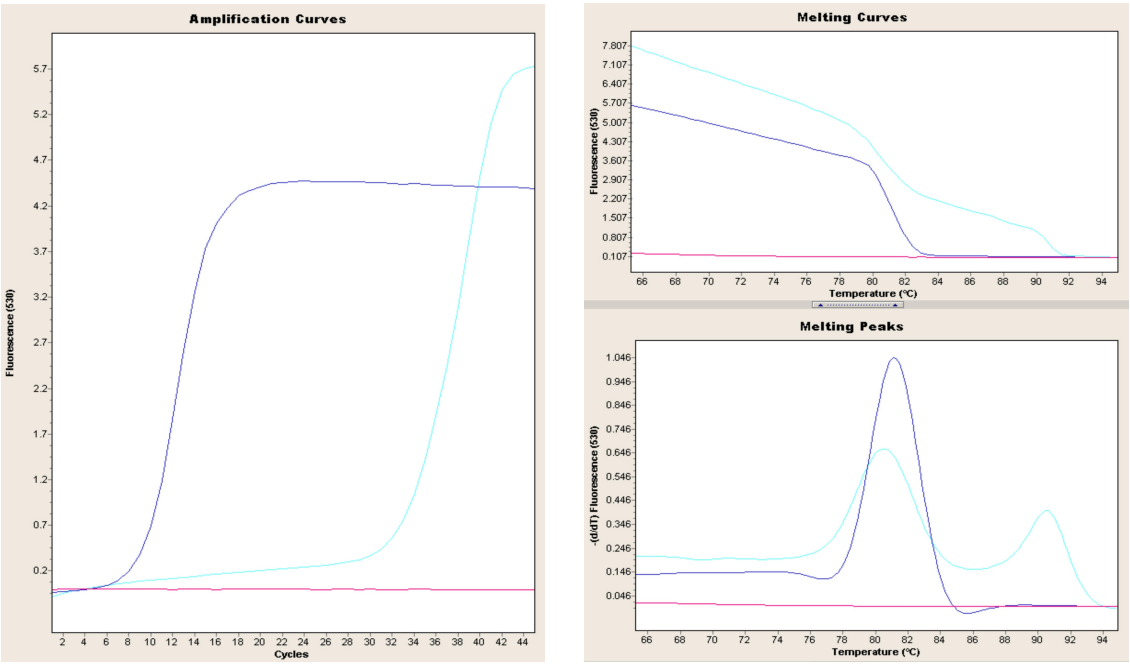

Figure V-6 Amplification and melting curve analysis. A typical example of amplification and melting curves comparing one horse sample (no. 72; turquoise; CP = 33.40; T_M = 80.93 °C) with a positive control (1:10 dilution of plasmid 30/7 (62, 63); blue; CP = 8.13; T_M = 81.29 °C), and a negative control (water; pink). In the negative control, no amplification occurred. The second peak in the horse sample represents formation of primer dimers. Data analysis was performed using LightCycler™ software (Roche).

Figure V-7

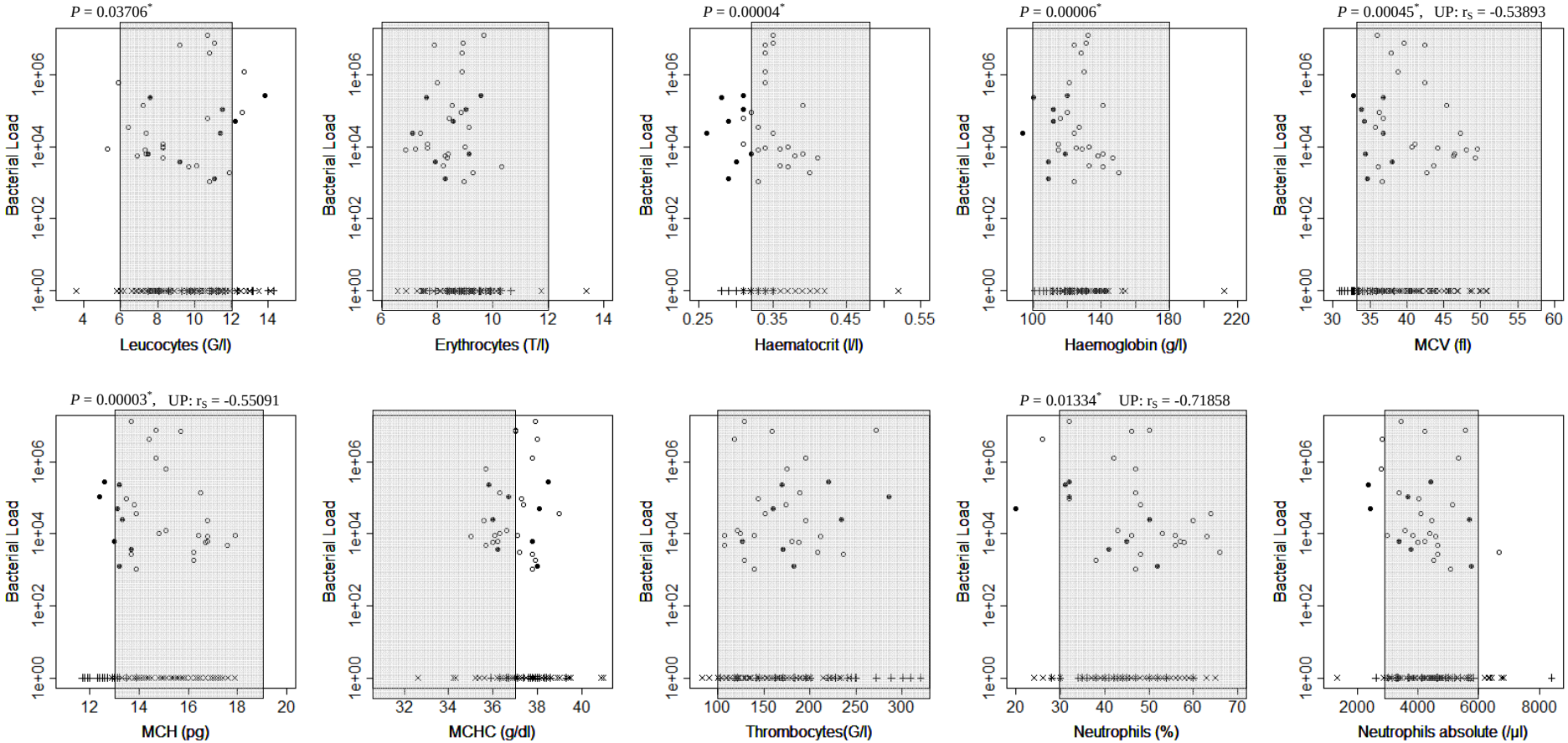
P = 0.03706*
Bacterial Load
Leucocytes (G/l)
Erythrocytes (T/l)
P = 0.00004*
Haematocrit (l/l)
P = 0.00006*
Haemoglobin (g/l)
P = 0.00045*, UP: rS = -0.53893
MCV (fl)
P = 0.00003*, UP: rS = -0.55091
MCH (pg)
MCHC (g/dl)
Thrombocytes(G/l)
P = 0.01334* UP: rS = -0.71858
Neutrophils (%)
Neutrophils absolute (/µl)

Figure V-7 continued

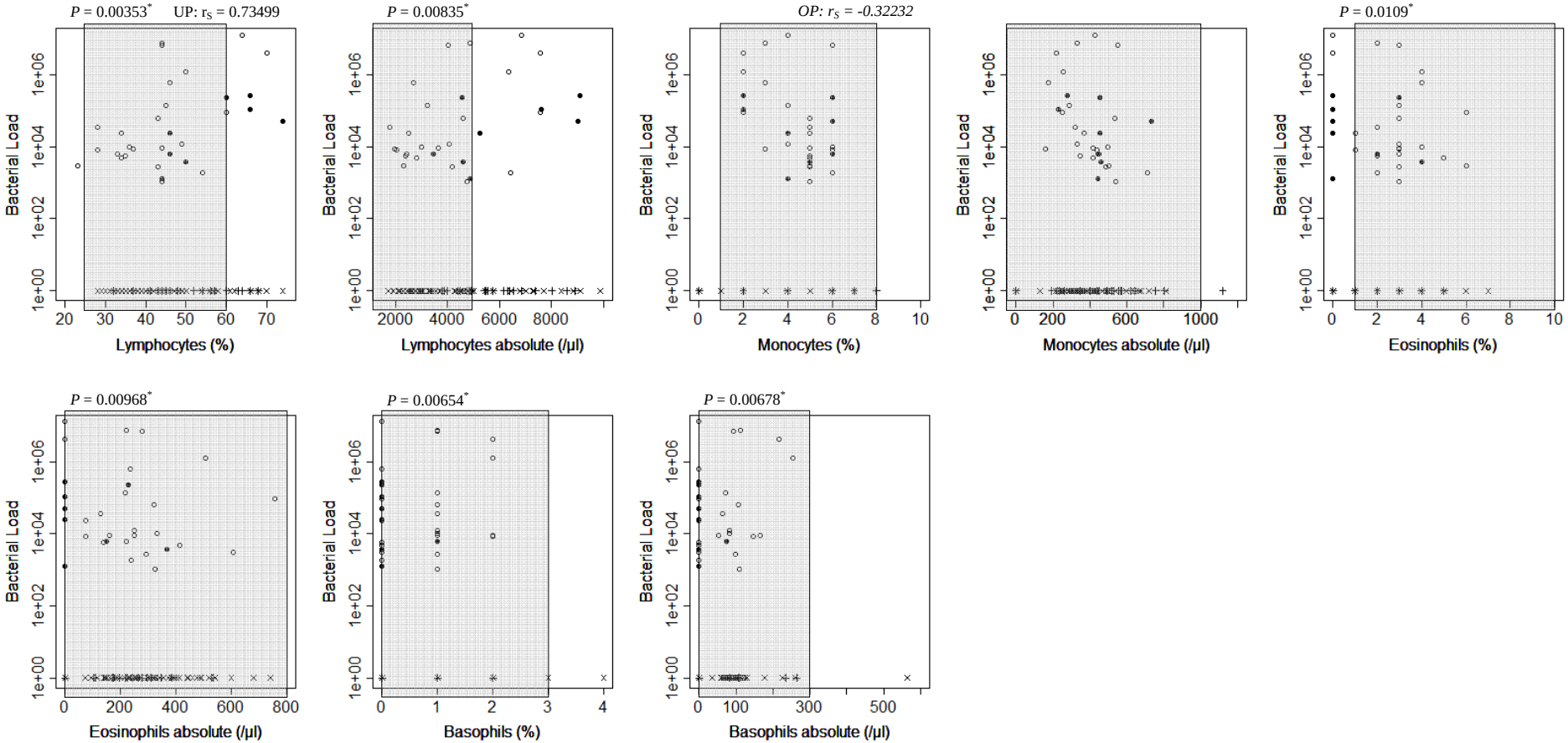

Figure V-7 Correlation of bacterial blood loads and haematological values. Bacterial blood loads (log copy number per mL of blood) were plotted against haematological values. 156 horses' blood counts were available. Fourteen horses' ages were unknown, and therefore they were excluded from analyses. Twenty-four samples re-collected one month later were also excluded. No samples exhibited an elevated number of segmented neutrophils or other cells. MCV = mean corpuscular haemoglobin, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration. Reference ranges (178) are indicated by a grey box. • = PCR positive horses older than one year, o = PCR positive horses younger than one year, x = PCR negative horses older than one year, + = PCR negative horse younger than one year. Scatterplots were generated using R Foundation for Statistical Computing (R version 2.13.0). Blood parameters of PCR positive horses older and younger than one year were compared using the one-sided Mann-Whitney *U*-Test. * = *P*-Values are outlined, if significant ($P \leq 0.05$). Correlation was analysed by the Spearman rank correlation coefficient (r_S). Only coefficients higher than 0.5 and lower than -0.5 are indicated. OP = PCR positive older than one year, UP = PCR positive younger than one year.

V.1.7 Comparison of microscopy and PCR results

Results of the SYBR green I real-time PCR assay were compared to microscopy of acridine orange and Giemsa stained peripheral blood smears (Tab. V-3, p. 67). Acridine orange stains were available from 164 samples, while only 158 samples were stained with Giemsa. Both stains were available from 118 samples. 211 samples were tested with the novel SYBR green I real-time PCR assay. Blood smears stained with either acridine orange or Giemsa were available from 204 samples. 102 of 204 samples showed signs of HM (50.0 %). In 37 of 158 Giemsa stained horse blood smears (23.4 %), HMs were detected. In acridine orange stained blood smears, HMs were detected in 80 of 164 samples (48.8 %). In fifteen of 118 samples (12.7 %), HMs were detected in Giemsa stained blood smears as well as in acridine orange stained blood smears. 72 of 118 samples (61.0 %) were negative in both staining techniques.

48 of 102 samples microscopically diagnosed as HM positive (either Giemsa or acridine orange stained) were confirmed positive by PCR (47.1 %). In 72 microscopically HM-negative samples, six reacted positive in PCR (8.3 %). This corresponds to a rate of false-positive microscopic results of 38.8 % and false-negative microscopic results of 9.2 %. Microscopy of Giemsa stained blood smears revealed in 37 samples HM, of which 17 reacted positive in PCR (45.9 %). 42 of 80 samples diagnosed as positive by microscopy of acridine orange stained blood smears reacted positive in PCR (52.5 %), corresponding to false-positive microscopic rates of 17.4 % in case of Giemsa staining and 34.2 % in case of acridine orange. The false-negative rates were 60.5 % (Giemsa) and 20.8 % (acridine orange). Overall, Giemsa staining and PCR results agree in 112

cases (70.9 %), acridine orange and PCR results yield the same result in 115 cases (70.1 %), and all three methods concur in 77 cases (65.2 %).

Table V-3 Correlation of microscopy and PCR results

Variable	Number of horses		
	Total	**PCR positive**	**PCR negative**
Microscopy (either Giemsa or acridine orange, n = 204)			
Positive	102	48	54
Negative	102	17	85
Giemsa (n = 164)			
Positive	37	17	20
Negative	121	26	95
Acridine orange (n = 158)			
Positive	80	42	38
Negative	84	11	73
Giemsa and acridine orange (n = 118)			
Positive in both staining techniques	15	11	4
Negative in both staining techniques	72	6	66
Positive in Giemsa, negative in acridine orange	2	0	2
Negative in Giemsa, positive in acridine orange	29	14	15

V.1.8 CARD-FISH

In the first trial, DAPI for nucleus staining was used as counterstain (Fig. V-8 A-C). Due to inefficient washing, the background from DAPI staining was very high, and even DNA-free RBCs appeared blue. Bacterial 16S rRNA was targeted by an universal EUB338 probe (7) labelled with HRP which catalysed the deposition of Cy5-labelled fluorescein tyramine. Detected bacteria appeared as red dots (Fig. V-8 C).

In a second trial, glycophorin A, which is a RBC membrane component, was targeted by a FITC-labelled antibody (Fig. V-8 D-F). DAPI staining was still too strong, so it was ignored for producing the overlay (Fig. V-8 F).

In both attempts, coccoid bacteria were detected on the RBC surfaces, as seen in the overlays (Fig. V-8 C & F, arrowheads). By hybridisation with a NONEUB probe (352), no structures on the RBC surface were detected (data not shown).

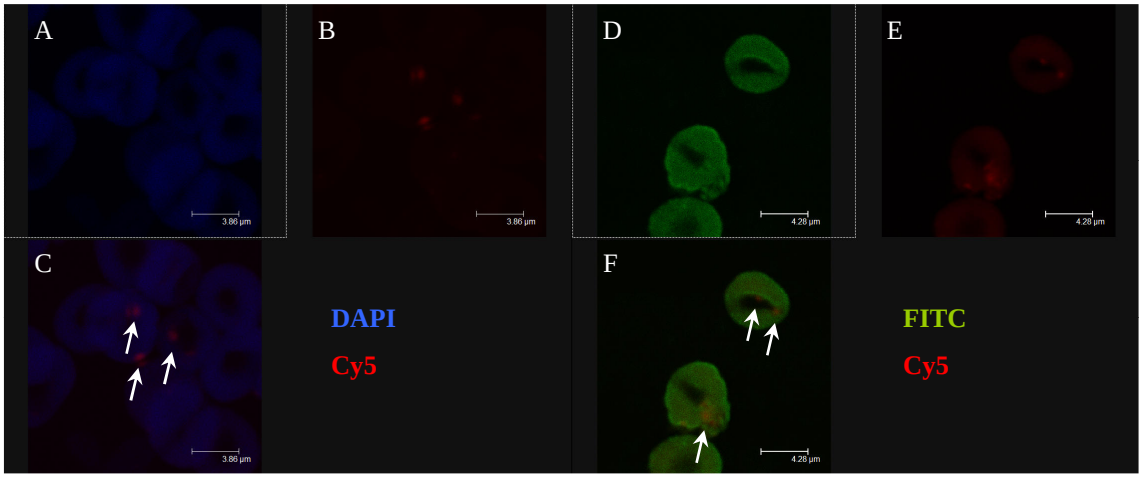

Figure V-8 Micrographs of CARD-FISH of horse blood infected with HM. DAPI (blue) was used as nucleus counterstain and glycophorin A (FITC, green) as the RBC membrane counterstain. Bacteria (white arrowhead) were specifically detected by a HRP-labelled EUB338 probe (red). HRP catalysed the deposition of Cy5-labelled fluorescein tyramine.

V.1.9 Description of the novel equine HM isolate: '*Candidatus* Mycoplasma equi'

'*Candidatus* Mycoplasma equi' [Latin: *equus*, horse] [(Mollicutes) NC; NA; O, wall less; NAS (GenBank acc. no. FN421445); S (horse, erythrocyte); DIECKMANN *et al.* Vet. Microbiol. 145: 351-353, 2010].

In Giemsa and acridine orange stained blood smears, coccoid bacteria (ca. 0.3 µm) were observed on the surface of equine RBCs. Microscopic pictures resembled other HM species (e.g. '*C*M turicensis' (367)). The novel HM species seems to be responsible for anaemia in horses, but exact clinical implications have not yet been determined. Analysis of partial 16S rRNA sequences showed that '*C*M equi' is most closely related to '*C*M haemobovis' and *M. haemofelis*.

V.1.10 Summary of results

Table V-4 Summary of results

No.	Date of sampling	Blood count				PCR		SYBR green		Bact. Load	Microscopy	
		Ht	Hb	RBCC	WBCC	16S	HM	CP	T_M		GI	AO
001	18.02.08	/	/	/	/	+	+	Neg			/	+
	16.11.09	0.37	137	8.80	7.2	-	-	Neg			-	+
002	18.02.08	/	/	/	/	+	-	**30.89**	**80.57**	**1.21×10^5**	/	+
	16.11.09	0.38	138	8.28	6.9	-	-	**33.66**	**80.59**	**5.65×10^3**	-	+
003	18.02.08	/	/	/	/	-	+	**30.86**	**80.83**	**1.30×10^5**	/	/
	16.11.09	0.33	115	6.54	7.7	-	+	Neg			+	+
	09.12.09	0.33	117	6.61	7.3	/	/	Neg			+	/
004	18.02.08	/	/	/	/	+	+	Neg			/	+
	16.11.09	0.36	133	8.22	10.1	+	-	**34.05**	**80.69**	**2.98×10^3**	-	+
005	18.02.08	/	/	/	/	+	+	Neg			/	+
	16.11.09	0.39	140	8.87	5.8	-	-	Neg			-	-
006	18.02.08	/	/	/	/	-	-	**32.62**	**80.18**	**5.80×10^6**	/	+
	16.11.09	0.40	141	8.91	8.0	+	-	Neg			-	-
007	18.02.08	/	/	/	/	-	+	**31.02**	**80.54**	**8.40×10^4**	/	+
	16.11.09	0.38	139	8.84	7.9	-	-	Neg			-	-
008	18.02.08	/	/	/	/	+	+	**29.65**	**80.72**	**4.36×10^7**	/	-
009	18.02.08	/	/	/	/	-	-	**31.73**	**80.69**	**1.06×10^7**	/	-
010	18.02.08	/	/	/	/	+	+	**28.95**	**80.90**	**7.05×10^7**	/	+
	16.11.09	0.32	118	8.29	6.0	-	-	Neg			-	+
011	18.02.08	/	/	/	/	-	-	Neg			/	+
	16.11.09	0.35	122	7.60	7.0	-	-	Neg			+	-
012	18.02.08	/	/	/	/	+	+	Neg			/	-
	16.11.09[§]	0.33	115	6.85	7.3	+	-	**32.63**	**80.86**	**8.25×10^3**	-	+
013	18.02.08	/	/	/	/	+	+	Neg			/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
014	18.02.08	/	/	/	/	+	+	**29.79**	**81.37**	**1.11×10^6**	/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
	16.11.09	0.36	129	7.20	5.3	+	-	**32.59**	**80.81**	**8.85×10^3**	-	+
015	18.02.08	/	/	/	/	-	+	**30.20**	**80.31**	**5.55×10^5**	/	+
	17.11.09	0.35	124	7.38	7.4	+	+	**32.05**	**80.46**	**2.35×10^4**	+	+
	09.12.09	0.33	118	6.98	7.0	/	/	**31.44**	**80.35**	**2.11×10^5**	-	/

Table V-4 continued

No.	Date of sampling	Blood count				PCR		SYBR green		Bact. Load	Microscopy	
		Ht	Hb	RBCC	WBCC	16S	HM	CP	T_M		GI	AO
016	18.02.08	/	/	/	/	+	+	Neg			/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
017	19.02.08	/	/	/	/	+	-	**32.13**	**81.06**	**8.05×10^6**	/	+
	15.05.08	/	/	/	/	+	+	**36.38**	**80.31**	**7.00×10^5**	/	+
	25.07.08	/	/	/	/	-	-	Neg			/	+
018	20.02.08	/	/	/	/	+	+	Neg			/	+
019	20.02.08	/	/	/	/	+	+	Neg			/	-
020	20.02.08	/	/	/	/	+	+	Neg			/	+
	18.11.09	**0.31**	115	7.64	8.3	-	-	**32.43**	**80.42**	**1.19×10^4**	+	+
	09.12.09	**0.31**	114	7.67	7.1	/	/	Neg			-	/
021	20.02.08	/	/	/	/	+	+	Neg			/	/
	16.11.09	0.39	143	8.54	11.0	+	-	Neg			-	-
022	20.02.08	/	/	/	/	+	+	**29.98**	**80.16**	**8.25×10^5**	/	+
	15.05.08	/	/	/	/	+	+	Neg			/	-
	16.11.09	0.37	133	9.00	8.3	-	-	**33.32**	**80.16**	**9.90×10^3**	-	-
023	20.02.08	/	/	/	/	+	+	**29.47**	**80.35**	**4.94×10^7**	/	-
	17.11.09	0.37	139	9.22	9.8	+	-	Neg			-	+
024	20.02.08	/	/	/	/	+	+	Neg			/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
	18.11.09	0.33	112	6.56	7.6	-	-	Neg			-	-
025	20.02.08	/	/	/	/	-	-	Neg			/	-
	16.11.09	0.39	140	9.27	6.8	/	/	Neg			-	-
026	20.02.08	/	/	/	/	+	+	**26.52**	**80.23**	**3.69×10^8**	/	-
	18.11.09	0.36	142	8.39	9.7	/	/	Neg			-	-
027	20.02.08	/	/	/	/	+	-	**29.82**	**81.06**	**3.89×10^7**	/	-
028	20.02.08	/	/	/	/	+	+	**29.57**	**80.21**	**4.62×10^7**	/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
029	20.02.08	/	/	/	/	+	+	**27.90**	**80.60**	**1.63×10^8**	/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
	18.11.09	0.36	129	7.42	8.1	/	/	Neg			-	-
030	20.02.08	/	/	/	/	+	+	**27.95**	**80.71**	**9.50×10^6**	/	+
	25.07.08	/	/	/	/	+	-	**29.62**	**80.98**	**1.41×10^6**	/	+
	18.11.09	0.41	147	8.36	8.3	/	/	**32.93**	**81.01**	**4.86×10^3**	-	-
031	20.02.08	/	/	/	/	+	+	**30.14**	**80.99**	**2.00×10^7**	/	+
	17.11.09	0.42	138	8.35	8.0	/	/	Neg			-	+
032	20.02.08	/	/	/	/	+	+	**28.09#**	**80.50#**	**$1.05 \times 10^{8\#}$**	/	+
	15.05.08	/	/	/	/	+	+	Neg			/	+
033	20.02.08	/	/	/	/	+	-	**29.30**	**80.71**	**4.40×10^7**	/	/
034	20.02.08	/	/	/	/	+	+	**28.87**	**80.04**	**6.55×10^7**	/	/
036	20.10.08	/	/	/	/	+	+	**30.68**	**80.62**	**2.03×10^5**	/	+
	17.11.09§	0.33	124	8.94	10.8	/	/	**34.69**	**80.67**	**1.05×10^3**	-	+
042	20.11.08	/	/	/	/	+	-	Neg			/	/
043	08.12.08	/	/	/	/	+	+	Neg			/	+
044	08.12.08	/	/	/	/	+	-	**34.98**	**80.33**	**2.18×10^5**	/	/
045	n/a	/	/	/	/	+	-	Neg			/	+
046	n/a	/	/	/	/	/	/	/			/	-
047	n/a	/	/	/	/	+	+	**33.63**	**82.07**	**7.70×10^5**	/	+
048	16.11.09	0.36	132	8.84	8.2	/	/	Neg			-	-
049	16.11.09	0.39	141	8.54	7.2	+	+	**31.07**	**80.94**	**1.38×10^5**	+	+
	09.12.09	0.39	142	8.62	6.8	/	/	**35.86**	**80.74**	**9.65×10^4**	+	/
050	16.11.09	0.39	141	8.37	7.4	/	/	**32.78**	**80.30**	**6.30×10^3**	-	-
051	16.11.09§	0.38	141	8.62	11.5	/	/	Neg			-	-
052	16.11.09	0.37	126	7.25	7.8	/	/	Neg			-	-

Table V-4 continued

No.	Date of sampling	Blood count				PCR		SYBR green		Bact. Load	Microscopy	
		Ht	Hb	RBCC	WBCC	16S	HM	CP	T_M		GI	AO
053	16.11.09§	0.35	127	7.46	7.5	/	/	Neg			-	-
054	16.11.09	0.38	144	9.12	8.7	/	/	Neg			-	-
055	17.11.09	**0.30**	110	7.91	9.3	/	/	Neg			-	-
056	17.11.09	**0.31**	118	9.90	10.3	/	/	Neg			-	-
057	17.11.09	**0.26**	**94**	7.09	11.4	-	+	**32.03**	**80.71**	**2.45×10^4**	+	+
	09.12.09	**0.31**	112	8.30	**15.0**	/	/	Neg			+	/
058	17.11.09	0.32	120	9.55	9.8	/	/	Neg			-	-
059	17.11.09	**0.29**	109	8.28	11.1	+	-	**32.17**	**80.60**	**1.28×10^3**	-	+
	09.12.09	**0.28**	105	7.94	**16.7**	/	/	**34.04**	**80.52**	**3.05×10^3**	+	/
060	17.11.09	**0.31**	120	9.55	**13.8**	+	+	**30.71**	**80.78**	**2.65×10^5**	+	+
	09.12.09	0.34	126	10.05	**16.3**	/	/	**35.71**	**80.16**	**2.00×10^2**	-	/
061	17.11.09	**0.31**	123	9.91	9.8	/	/	Neg			-	-
062	17.11.09	**0.28**	100	7.58	7.6	+	-	**30.79**	**80.64**	**2.29×10^5**	-	+
	09.12.09	0.34	126	9.58	**12.2**	/	/	Neg			-	/
063	17.11.09	**0.31**	114	9.53	9.6	-	-	Neg			+	+
	09.12.09	0.35	130	10.8	**13.4**	/	/	Neg			-	/
064	17.11.09	**0.30**	112	8.86	11.7	/	/	Neg			-	+
	09.12.09	0.32	117	9.04	**17.0**	/	/	Neg			-	/
065	17.11.09	0.32	123	10.25	10.3	/	/	Neg			-	-
066	17.11.09	**0.30**	112	9.04	**13.1**	/	/	Neg			-	-
067	17.11.09	**0.31**	112	9.03	11.5	-	+	**32.37#**	**81.49#**	**$6.11 \times 10^{4\#}$**	+	+
	09.12.09	0.33	120	9.47	**12.9**	/	/	**35.81**	**80.27**	**1.70×10^2**	-	/
068	17.11.09	0.33	122	9.31	10.0	/	/	Neg			-	-
069	17.11.09	**0.30**	109	7.93	9.2	+	+	**33.55**	**81.42**	**3.73×10^3**	+	+
	09.12.09	0.32	116	8.45	9.1	/	/	Neg			-	/
070	17.11.09	**0.28**	104	7.72	8.6	/	-	Neg			+	-
	09.12.09	0.35	130	9.84	11.5	/	/	**34.73**	**81.58**	**9.80×10^2**	-	/
071	17.11.09	**0.30**	111	8.43	8.1	/	/	Neg			-	-
	09.12.09	**0.30**	106	8.16	**12.6**	/	/	Neg			-	/
072	17.11.09	**0.29**	111	8.95	**14.3**	/	+	Neg			-	+
	09.12.09	0.32	119	9.57	**19.5**	/	/	**35.40**	**80.98**	**3.31×10^2**	+	/
073	17.11.09	0.33	122	8.07	6.6	/	/	Neg			-	-
075	17.11.09	0.37	133	8.50	6.2	/	/	Neg			-	+
076	17.11.09	0.35	128	7.86	7.1	/	/	Neg			-	+
077	17.11.09	0.37	135	8.71	10.6	/	/	Neg			-	+
078	17.11.09	0.38	141	8.42	7.7	/	/	Neg			-	-
079	17.11.09	0.37	143	9.02	8.9	/	/	Neg			-	-
080	17.11.09§	0.39	137	8.68	10.2	/	/	Neg			-	-
081	17.11.09	0.36	133	8.91	9.3	/	/	Neg			-	-
082	17.11.09	0.32	123	8.92	7.1	/	/	Neg			-	-
083	17.11.09§	0.34	131	9.22	11.1	/	/	Neg			-	-
084	17.11.09	0.36	132	9.58	**12.5**	/	/	Neg			-	-
085	17.11.09	0.35	131	8.93	11.1	+	-	**30.25**	**80.85**	**7.45×10^6**	+	+
	09.12.09	0.33	122	8.27	9.4	/	/	**34.53#**	**81.22#**	**$1.18 \times 10^{6\#}$**	-	/
086	17.11.09	**0.31**	115	7.53	7.2	/	/	Neg			-	-
087	17.11.09	0.33	128	9.28	10.6	/	/	Neg			-	-
088	17.11.09	0.34	132	9.11	**12.8**	/	/	Neg			-	-
089	17.11.09	0.34	131	9.48	**14.1**	/	/	Neg			-	-
090	17.11.09	0.34	125	7.62	8.3	+	+	**33.07**	**80.94**	**8.95×10^3**	+	+
	09.12.09	**0.31**	111	6.82	6.4	/	/	Neg			-	/
091	17.11.09	0.37	140	8.09	7.5	/	/	Neg			-	+
092	17.11.09	**0.31**	115	7.50	9.3	/	/	Neg			-	-
093	17.11.09	0.41	152	8.49	10.5	/	/	Neg			-	-

Table V-4 continued

No.	Date of sampling	Blood count				PCR		SYBR green		Bact. Load	Microscopy	
		Ht	Hb	RBCC	WBCC	16S	HM	CP	T_M		GI	AO
094	18.11.09	0.52	212	13.38	3.6	/	/	Neg			-	-
095	18.11.09	0.32	117	8.07	7.7	/	/	Neg			-	-
096	18.11.09	0.34	128	8.89	10.8	/	/	**30.78**	**81.01**	**4.15×10^6**	-	+
097	18.11.09	0.38	154	10.26	9.9	/	/	Neg			-	-
098	18.11.09	0.32	120	7.83	11.1	+	+	Neg			+	+
	09.12.09	0.36	135	8.99	9.2	/	/	**33.65**	**81.97**	**7.60×10^5**	-	/
099	18.11.09	0.34	121	6.87	8.1	/	/	Neg			-	-
100	18.11.09	0.36	137	9.73	10.0	/	/	Neg			-	-
101	18.11.09	0.37	140	9.88	9.6	/	/	Neg			-	-
102	18.11.09	0.33	126	8.98	13.5	/	/	Neg			-	-
103	18.11.09	0.34	124	7.90	9.2	-	+	**29.43**	**81.09**	**6.70×10^6**	+	+
	09.12.09	0.34	123	7.87	8.7	/	/	Neg			-	-
104	18.11.09	0.39	137	8.80	10.3	/	/	Neg			-	-
105	18.11.09	0.31	116	8.42	10.7	-	+	**34.54**	**81.54**	**6.30×10^4**	+	+
106	18.11.09	0.34	121	7.99	5.9	/	/	**32.49**	**81.05**	**6.15×10^5**	-	-
107	18.11.09	0.32	123	9.56	11.7	/	/	Neg			-	-
108	18.11.09	0.35	132	9.66	10.7	/	/	**29.07**	**80.84**	**1.27×10^7**	-	-
109	18.11.09	0.33	120	8.82	7.4	/	/	Neg			-	-
110	18.11.09	0.32	123	8.54	8.6	/	/	Neg			-	-
111	18.11.09	0.33	127	9.14	6.4	/	/	**32.32**	**81.28**	**3.50×10^4**	-	-
112	18.11.09	0.34	127	8.40	8.5	/	/	Neg			-	-
113	18.11.09	0.38	142	9.20	9.3	/	/	Neg			-	-
114	18.11.09	0.40	150	9.27	11.9	/	/	**33.93**	**80.96**	**1.87×10^3**	-	+
115	18.11.09	0.36	136	9.99	10.6	/	/	Neg			-	-
116	18.11.09	0.37	141	10.32	9.7	/	/	**33.73**	**81.17**	**2.69×10^3**	-	+
117	18.11.09	0.35	133	9.31	11.3	/	/	Neg			-	-
118	18.11.09	0.32	120	8.28	10.3	+	+	Neg			+	+
119	18.11.09	0.35	138	10.11	11.9	/	/	Neg			-	-
120	18.11.09	0.35	136	9.89	10.0	/	/	Neg			-	-
121	18.11.09	0.34	130	8.44	13.0	/	/	Neg			-	-
122	18.11.09	0.37	137	9.24	12.3	/	/	Neg			-	+
123	18.11.09	0.35	131	9.83	10.9	/	/	Neg			-	-
124	18.11.09	0.36	141	10.13	11.4	/	/	Neg			-	-
125	18.11.09	0.35	132	8.87	11.5	/	/	Neg			-	-
126	18.11.09	0.34	130	8.87	12.7	/	/	**30.28**	**80.51**	**1.24×10^6**	-	+
127	18.11.09	0.32	120	8.86	12.6	/	/	**31.78**	**80.70**	**9.25×10^4**	-	+
128	18.11.09	0.39	154	11.75	13.0	/	/	Neg			-	-
129	18.11.09	0.38	140	8.12	6.6	/	/	Neg			-	-
130	18.11.09	0.36	136	8.55	5.8	/	/	Neg			-	-
131	19.11.09	0.29	108	8.76	10.9	+	+	Neg			-	-
	09.12.09	0.32	120	9.64	10.7	/	/	Neg			+	/
132	19.11.09	0.29	112	8.57	12.2	-	+	**32.12**	**81.59**	**5.05×10^4**	-	+
	09.12.09	0.33	126	9.60	12.7	/	/	Neg			-	/
133	19.11.09	0.33	126	10.64	12.3	/	/	Neg			-	-
134	19.11.09	0.35	131	10.65	11.2	/	/	Neg			-	-
135	19.11.09[§]	0.29	106	8.34	10.8	+	+	Neg			-	+
	09.12.09	0.30	107	8.25	13.8	/	/	Neg			+	/
136	19.11.09	0.33	123	9.52	13.2	/	/	Neg			-	+
	09.12.09	0.34	125	9.49	15.1	/	/	Neg			-	/
137	19.11.09	0.34	129	10.29	11.3	/	/	Neg			-	-
138	19.11.09	0.33	124	9.75	11.5	/	/	Neg			-	-
139	19.11.09	0.32	116	9.02	14.0	/	/	Neg			-	+
140	19.11.09	0.32	119	9.15	7.5	/	/	**33.60**	**80.88**	**6.20×10^3**	-	+

Table V-4 continued

No.	Date of sampling	Blood count				PCR		SYBR green		Bact. Load	Microscopy	
		Ht	Hb	RBCC	WBCC	16S	HM	CP	T_M		GI	AO
141	19.11.09	**0.31**	119	9.15	10.7	/	/	Neg			-	-
142	19.11.09	0.32	121	9.50	8.7	/	/	Neg			-	+
143	06.01.10	0.35	125	7.61	6.1	+	+	**33.15**[#]	**81.21**[#]	**n.d.**	+	/
144	06.01.10	0.32	115	6.75	6.6	-	-	Neg			+	/
145	29.12.09	**0.15**	**53**	**3.00**	8.5	-	-	Neg			+	/
146	29.12.09	**0.25**	**91**	**5.00**	5.1	-	-	Neg			+	/
147	16.12.09	**0.26**	**98**	6.11	8.5	-	-	Neg			+	/
148	30.12.09	**0.28**	101	8.62	**14.3**	-	-	Neg			-	/
149	10.12.09	0.32	114	6.83	**13.3**	-	-	Neg			+	/
150	28.12.09	**0.29**	105	6.88	**30.4**	-	-	Neg			-	/
151	17.12.09	**0.29**	105	**5.80**	5.5	-	-	Neg			-	/
152	24.01.10	**0.31**	115	6.57	**2.6**	-	-	Neg			-	/
153	19.01.10	0.33	118	7.52	10.2	-	-	Neg			-	/
154	19.01.10	0.40	132	7.84	6.4	-	-	Neg			+	/
155	17.01.10	**0.27**	**94**	6.14	6.0	-	-	Neg			+	/
156	21.01.10	**0.31**	**96**	**5.75**	**4.6**	-	-	Neg			+	/
157	24.01.10	**0.20**	**69**	**4.90**	**19.6**	-	-	Neg			+	/
158	n/a	/	/	/	/	-	-	Neg			+	/
159	n/a	/	/	/	/	+	+	**35.72**	**81.38**	**n.d.**	+	/
160	n/a	/	/	/	/	/	/	**33.04**	**81.32**	**n.d.**	/	/
161	n/a	/	/	/	/	+	+	**31.77**[#]	**81.30**[#]	**2.79 x 10^7**[#]	+	/

No. = sample number; [§] = horses with a preliminary report of clinical sings characteristic of HM: meagre appearance, shaggy fur, and reduced performance; Ht = haematocrit (L/L, reference range: 0.32-0.48), Hb = haemoglobin (g/L, reference range: 100-180), RBCC = red blood cell count (10^{12} cells/L, reference range: 6.0-12.0), WBCC = white blood cell count (10^9 cells/L, reference range: 6.0-12.0); CP = crossing point; T_M = melting temperature; Neg = negative; Bact. load = bacterial load (per mL of blood); [#] samples were tested twice in SYBR green I real-time PCR and then mean values of CP, T_M, and bacterial load were calculated; n.d. = not determined; GI = Giemsa stain; AO = acridine orange stain; - = negative; + = positive; / = not available; n/a = not available. Positive PCR and microscopy results are highlighted in red boldface, decreased blood parameters in blue boldface, and increased blood parameters in orange boldface.

V.2 Phylogenetic analysis of haemotrophic mycoplasmas

V.2.1 General

All calculated trees showed similar topologies. A typical tree is presented in Figure V-9 (p. 77). In concordance with previously published 16S rRNA phylogenies of haemotrophic mycoplasmas (155, 220), HM are closely related to the 'pneumoniae-group', and their closest relatives are members of the 'fastidiosum-cluster' (*M. fastidiosum*, *M. cavipharyngis*, and *M. insons*). Within the HM cluster, two subclusters were observed. These two subclusters were termed 'haemofelis-group' and 'haemominutum-group' by PETERS *et al.* (243). This terminology is employed in this thesis as well. The 'haemofelis-group' comprises the HM species *M. haemofelis*, *M. haemocanis*, *M. coccoides*, *M. haemomuris*, and the rather novel isolates '*C*M turicensis' and '*C*M haemobovis'. The well-known species *M. ovis*, *M. wenyonii*, and *M. suis* belong to the 'haemominutum-group' just like the novel isolates '*C*M erythrocervae', '*C*M erythrodidelphis', '*C*M kahanei', '*C*M haemozalophi', '*C*M haemolamae', '*C*M haemominutum', and '*C*M haematoparvum'. Differences of the 16S rRNA sequences of the two subclusters are outlined in Table V-5.

Two-dimensional models of secondary structures of 16S rRNA were created. Within the subclusters, the overall architecture of 16S rRNA secondary structure is rather similar except for minor base substitutions, deletions, and insertions. However, structures of the two HM subclusters differ in four certain aspects. Figures V-10 & 11 (pp. 78-79) show the representative structures of the 'haemofelis'- and 'haemominutum-group' (*M. haemofelis* and *M. wenyonii*), respectively. The members of the 'haemofelis-group' exhibit shorter versions of loops eleven and eighteen, whereas loops six and ten are longer.

Table V-5 Nucleotide positions characteristic for the two haemotrophic Mycoplasma subclusters

Pos	Hfg	Hmg	Fsg	Png	Exceptions Hfg	Hmg
114	U	C	U	U		
132	A	U	C	A, C, U		Cmk: C
140	C	A, G	A	U	cap, Mhm: A; Mcc: U	
146	G	A	A, G	G		Cme: G
154	C	A	C, U	U	Mhm: U	Cmz: U
167	G	U	A, G	A	Mhm: A	Cmz: G
176	U	C	C, U	C		
177	C	A, C, U	U	C, G, U		
188	A, C, U	A, G	C, T	C		
190	A	-	A	U	Mhf/ Mhc: U	Cmz: U; Cml: A
191	G	-	G	G	cap, Mhm: U	Cmz: U; Cmm, Cmp: C
199	C, G	A, G	A, G	A, G, T		

Table V-5 continued

Pos	Hfg	Hmg	Fsg	Png	Exceptions Hfg	Hmg
200	-	A, G	C, U	A, U		Cmo: U, Cmk: C
201	C, U	G	C, G	C, G	Mcc: A	
202	G, U	C	C	C		
222	U	G	U	U	Cmt, cap: U	
248	C, U	A, G	A	A, C		
276	G	C			cap, Mcc: A	Msu: C, U
284	A	G	G	G		
286	A	G	A	G	Cmt: G	Cmo: A
294	U	A	A	G		Cmk, Cmp: G
297	U	G			Cmt, Mhm: A	
303	A	U	U	C, U		Cmp: C
313	A	G	A	A		
359	A	G	A	A		
360	G	A	G	G		
366	C	U	U	U		
369	C	U	C	U		
403	U	C	C	C		Cmh: U
444	A, G	U	A	A, G, U		
445	G	A	G	G		
453-481		25 bp trunc.				Cmo: 17 bp trunc. (458-474)
490	C, U	A	U	A, C, U		
508	A	U	U	A, U		
518	U	A	U	U		Cme, Cmk: C
544	G	A	G	A, G		
555	T	C	C	C		
576	C	G	C	A, C, G		
582	C	U	C	C		
590	U	A, C, G	G, U	U		
595	A	U	A	A		Cmv: C, Msu: G
596	A	G	A	A		Msu: U
597	G	A	G	G		
599	U	C	C	C		
601	U	A	A, G	G		
602	G	U	G	G		Cmk, Cmz: G
614	G	C, U	A, G	A, G, U		
636	C	A, U	U	U		Msu, Cme: G
637	C	U	U	U	Mhm: A	
644	U	C	U	U		Msu: U
658	G	U	A	A, G, U	Mhm: A	
659	U	A	C	U	Mhm: C	
661	G	U	G	A, G		
667	A	G	A	A		
670	U	A	U	U		
671	U	C	C	C, U		
679	A	C	C	C	Mhm: G	
683	A	G	G	G	Mhm: G	
699	U	C	C	C		
711	U	G	G	G	Mhm: C	
735	A	G	G	A, G		

Table V-5 continued

Pos	Hfg	Hmg	Fsg	Png	Exceptions Hfg	Hmg
736	A	U	A	A		
739	U	C	U	U		
744	C	A	C	C, U		
770	U	C	U	U		
830	A	G	U	A, G	Cmb/ Cmq, Mcc: G	Msu: U
835	G	U	G	G	Mhf/ Mhc: A	
851	C	G	C	C	Mhf/ Mhc: U	
856	U	C	A	C, U	Cmb/ Cmq, Mcc: C	Msu: A
864	U	A	A	A	Cmb/Cmq: A	
893	C	U	C	C	cap: U	
917	G	A	G	G		
990	U	C	U	C, U		
994	A	C, U	A	A		
995	C	A, U	C	C		
999	C	U	C	C, U		
1000	C, U	A, U	C	U		
1040	G	A, U	G	A	Cmb/ Cmq, Cmt: A	
1100	C	U	C	C		
1145	U	A	U	U		
1147	-	U	-	A, G		
1155	A	U	A	A		Msu: A, U
1164	C	U	G	A, G		
1165	A	C	C	C		
1171	U	G	G	G		
1172	G	A	C	C, U		
1188	A	G	A	A		
1189	U	C	U	U		
1192	C	A	C	C		Cmv: G
1271	G	A	G	G	Mcc: A	
1274	A	G	A	A, G	Cmb/ Cmq: G	
1292	C, U	C	C	C, U		
1293	C, U	U	A	G		
1327	U	A	U	U	Mhf/ Mhc: C	
1354	C	U	C	C		Msu: C, U
1368	G	A	G	G		
1377	A, G	A	A	A	Mhf/ Mhc: C	Cme, Cmh: G
1424	G	U	G	U	Mhm: A	Cml: A
1425	G	A	U	A		
1426	G	C	A	A		Mov/ Cmo: U
1429	U	A, G	U	C, U		
1436	A	C	C	A, C		

Numbering of positions (pos) correspond to the numbering of the 16S rRNA sequence of *E. coli*. Only positions, in which the consensus sequence of 'haemofelis' (Hfg)- and 'haemominutum' (Hmg)-group differs, were taken into consideration; Fsg = 'fastidiosum-group', Png = 'pneumoniae'-group; Mhm = *M. haemomuris*, Cmt = '*C*M turicensis', Msu = *M. suis*, Cmm = '*C*M haemominutum', Cmp = '*C*M haematoparvum', Cmk = '*C*M kahanei', cap = novel HM isolates from capybara, Mhf = *M. haemofelis*, Mhc = *M. haemocanis*, Cme = '*C*M erythrodidelphis', Mcc = *M. coccoides*,

Cmz = '*C*M haemozalophi', Cml = '*C*M haemolamae', Cmb = '*C*M haemobovis', Cmq = '*C*M equi', Mwe = *M. wenyonii*, Cmo = '*C*M haemovis', cer = novel HM isolates from cervids, Cmv: '*C*M erythrocervae', Mov = *M. ovis*; trunc. = truncation; A = adenine, C = cytosine, G = guanine, U = uracil, - = gap.

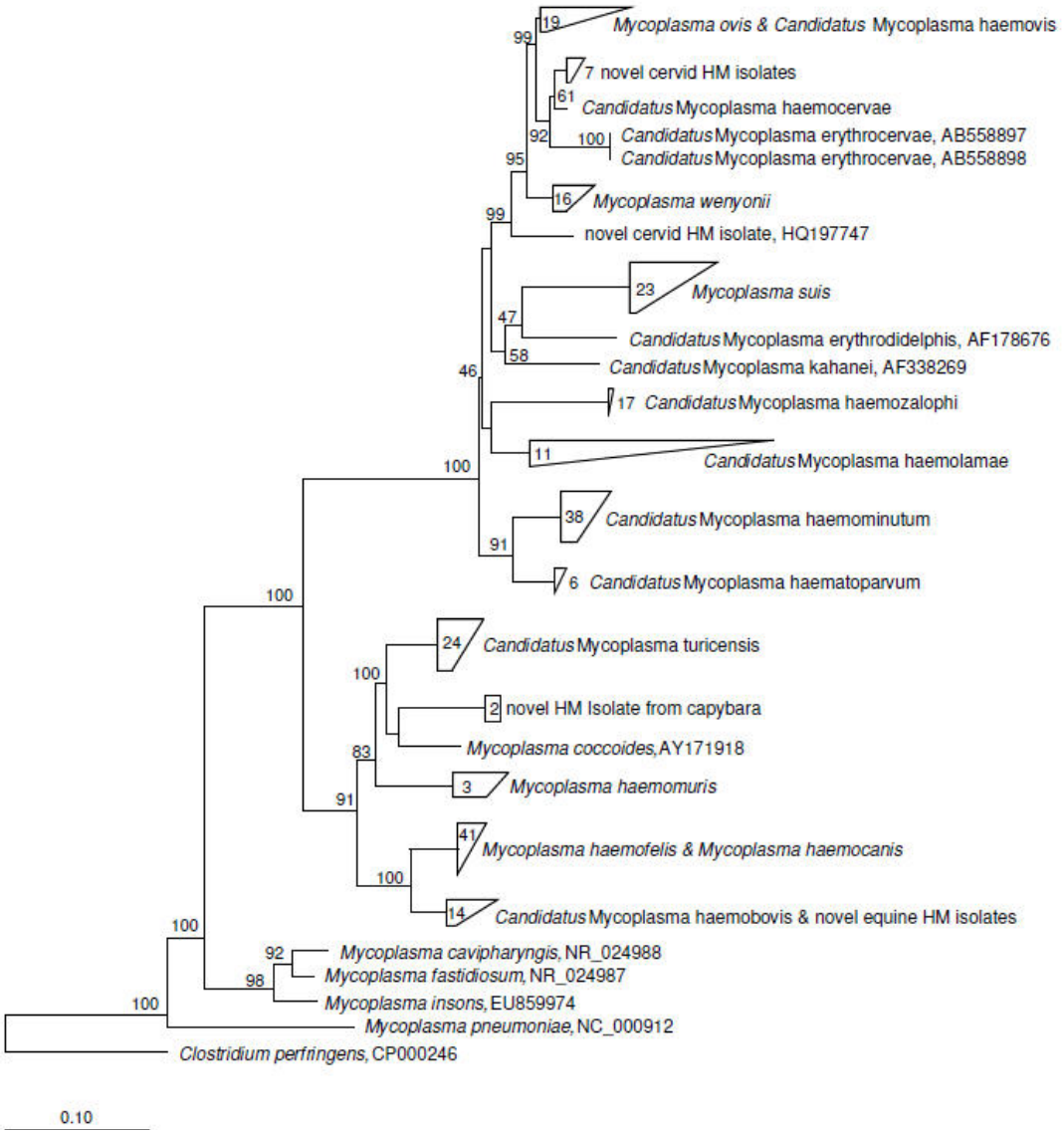

Figure V-9 Phylogenetic tree of haemotrophic mycoplasmas based on 16S rRNA sequences. A tree calculated by the maximum likelihood method in combination with a similarity filter of 25 % is shown. The tree was re-sampled 1000 times. Bootstrap percentage values are given as numbers at the nodes. Only values higher than 45 % are shown. The scale bar indicates the estimated evolutionary distance. Species and GenBank acc. no. are given at axes of single isolates. Clusters including more than two species were grouped. The triangle size indicates homology within the clusters, and the number inside of the triangle indicates the number of group members. *Clostridium perfringens* served as outgroup for tree rooting. The alignment of one member of each species can be found in the appendix on pp. 194-201.

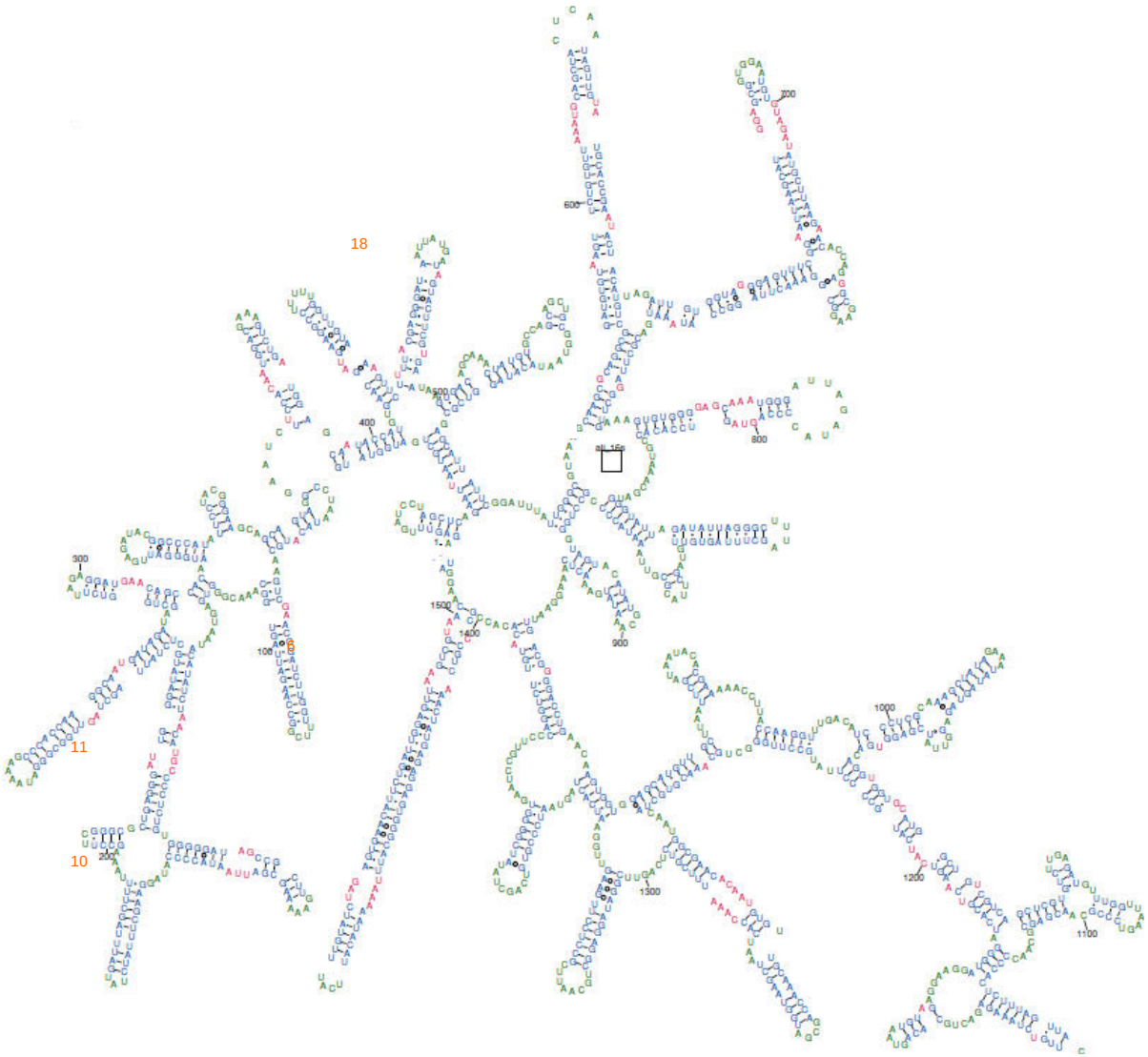

Figure V-10 Two dimensional model of 16S rRNA secondary structure of *M. haemofelis* (NC_014970, 1429 bp). Structure was created using the ARB software package (187) and processed with Xfig (http://www.xfig.org/). Colour code: blue = helix pairing, red = non-helix pairing, green = loop. Black numbers indicate base positions according to *E. coli* numbering, and orange numbers indicate helix numbers.

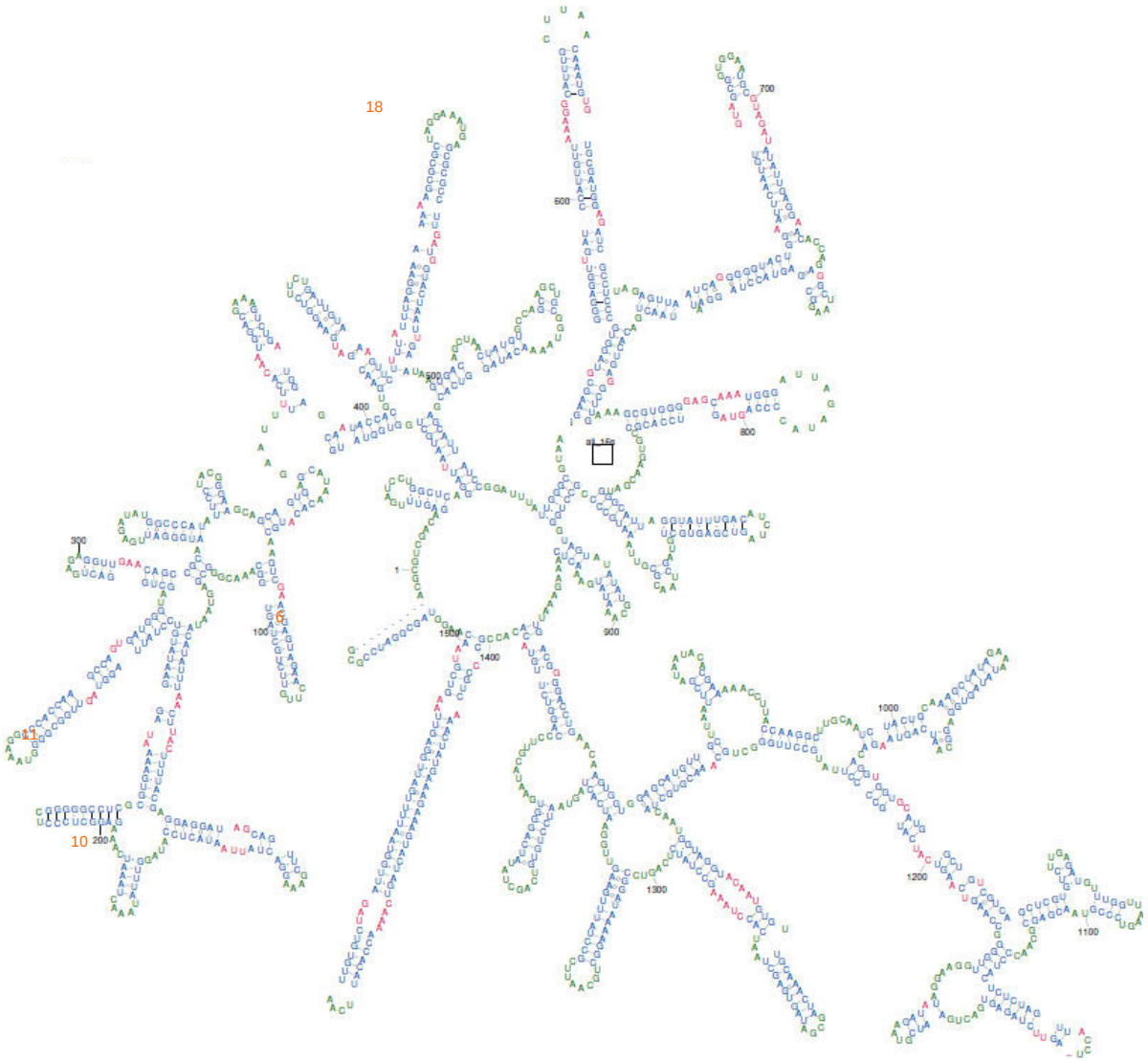

Figure V-2 Two dimensional model of 16S rRNA secondary structure of *M. wenyonii* (AY946266, 1471 bp). Structure was created using the ARB software package (187) and processed with Xfig (http://www.xfig.org/). Colour code: blue = helix pairing, red = non-helix pairing, green = loop. Black numbers indicate base positions according to *E. coli* numbering, and orange numbers indicate helix numbers.

In all 16S rRNA trees calculated in this study, *M. ovis* and '*C*M haemovis' could not be separated into two distinct clusters (Fig. V-12 A). '*C*M haemovis' was described in 2009 (140) and differs from *M. ovis* only by a 17 bp truncation in loop 18 (Fig. V-12 B & C) and some minor base substitutions (Tab. V-6).

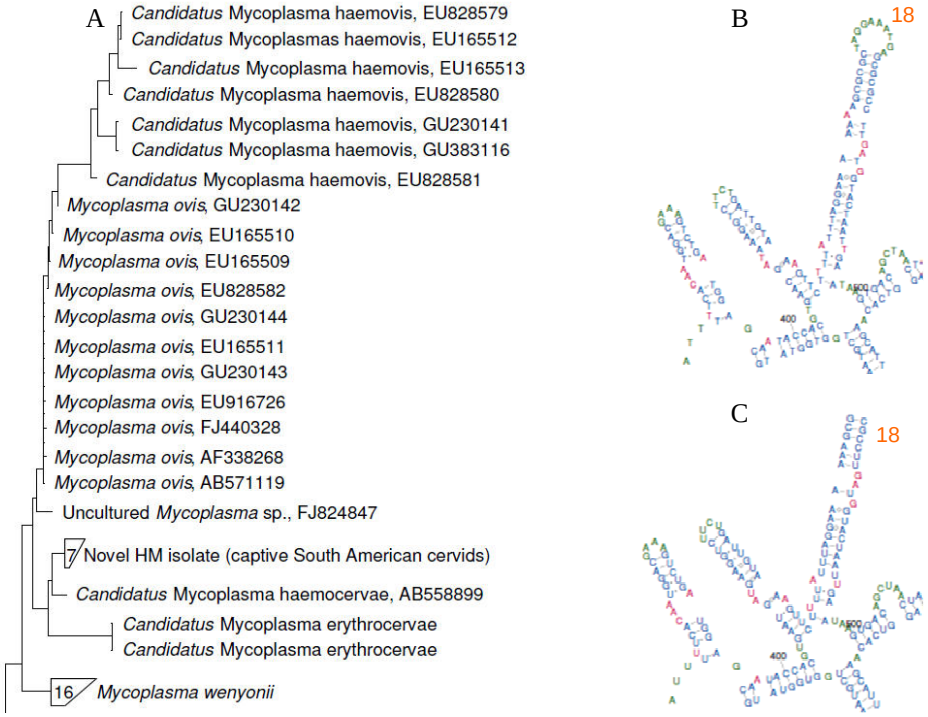

Figure V-12 Part of the 16S rRNA tree showing the position of *M. ovis* and '*C*M haemovis' and part of the 16S rRNA secondary structures. Part of a tree calculated with the maximum likelihood method and a 25 % similarity filter is shown in panel A. To see the whole tree, refer to Figure V-10, p. 77. Panel B shows part of the 16S rRNA secondary structure of *M. ovis* (GU230144), and panel C describes part of the 16S rRNA secondary structure of '*C*M haemovis' (EU165512). The overall structures of both species resemble the structure of *M. wenyonii* (Fig. V-11, p. 79).

Table V-6 Nucleotide positions characteristic for the ovine *Mycoplasma* isolates *M. ovis* (Mov) and '*C*M haemovis' (Cmo)

Pos	Cmo	Mov	Hmg	Hfg	Fsg	Png
200	U	G	A, G	-	C, U	A, U
286	A	G	G	A, G	A	G
354	A	G	G	G	G	G
458-475	17 bp trunc.					

Numbering of positions (pos) correspond to *E. coli*; Hmg = 'haemominutum'-group, Hfg = 'haemofelis'-group, Fsg = 'fastidiosum-group', Png = 'pneumoniae'-group; trunc. = truncation; A = adenine, C = cytosine, G = guanine, U = uracil, - = gap.

V.2.2 Phylogeny of novel HM isolates in cattle

In anaemic cattle in Northern Germany, two distinct HM isolates were found (Fig. V-13). One isolate was clearly identified as the well-known species, *M. wenyonii* (2, 219), and the other isolate was identified as the rather new species '*Candidatus* M. haemobovis' (139, 314). These results were published ((130); pp. 137-139).

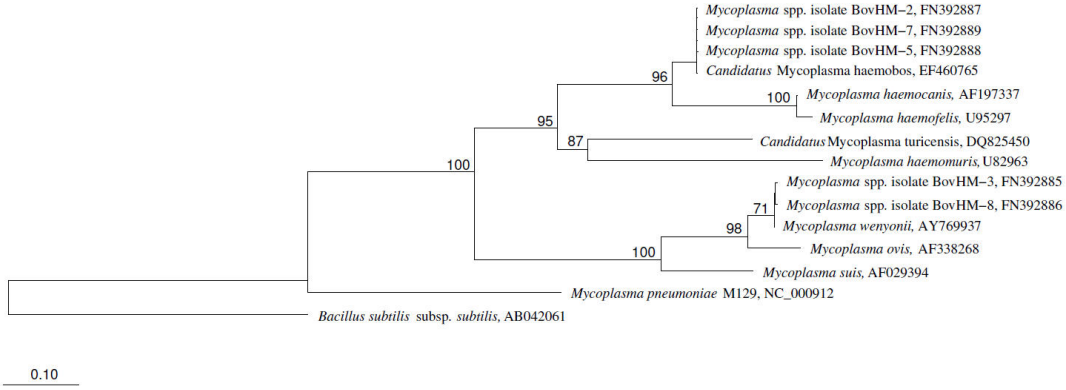

Figure V-3 Phylogenetic analysis of 16S rRNA gene sequences illustrating the position of the bovine isolates among the HM group. The tree was constructed using the maximum likelihood method and a minimum similarity filter of 25 %. Bootstrap values are given in percent at the tree nodes (1000 bootstraps). Species names and the corresponding acc. no. are given at each axis. The scale bar represents the estimated evolutionary distance. *Bacillus subtilis* served as an outgroup for tree rooting. The tree was reprinted from HOELZLE *et al.*, 2011 ((130), pp. 137-139). The corresponding alignment can be found in the appendix on pp. 202-206.

V.2.3 Phylogeny of Mycoplasma suis in wild boars

Occurrence of *M. suis* in wild boars was reported for the first time in Germany. A prevalence of 10.03 % was found. Characterisation of eighteen randomly chosen isolates revealed two *M. suis*-subclusters: the 'Guangdong'- and the 'Illinois'-cluster (Fig. V-14). These results were published ((128); pp. 129-133).

V.2.4 Phylogeny of the novel equine HM isolate

A novel equine HM isolate was found in Northern German horses. Partial 16S rRNA sequences (880 to 916 bp; acc. no. FN421443, FN421444, FN421445) showed the greatest sequence identity (97-98 %) with the bovine HM species '*C*M haemobovis' (Tab. V-7, p. 82; Fig. V-15, p. 83). Refer to DIECKMANN *et al.*, 2010 ((62); pp. 134-136) for more details.

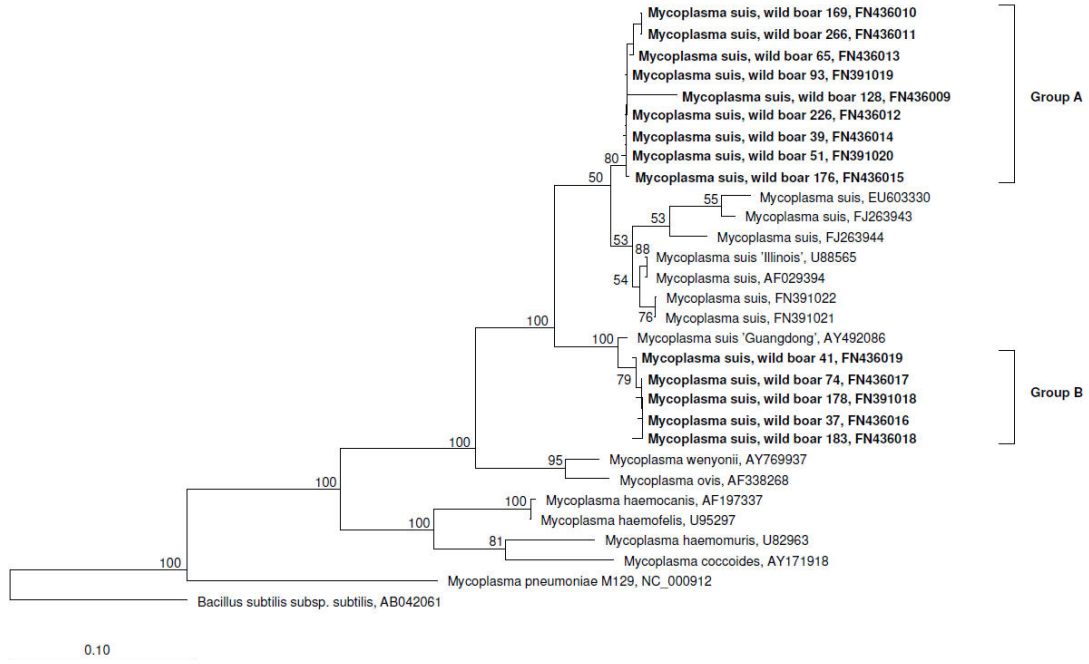

Figure V-4 Phylogenetic tree based on 16S rRNA sequences showing the position of the wild boar isolates among HMs. The tree was constructed using the neighbour-joining method in combination with the Felsenstein correction model and a 25 % similarity filter. Species assignation and accession numbers are given at each axis. *Bacillus subtilis* served as an outgroup. Bootstrap values are given at the nodes of the tree (1000 bootstraps). The scale bar indicates estimated evolutionary distance. The wild boar isolates are highlighted in bold face. They form two groups, group A showing highest similarity to *M. suis* 'Illinois' (U88565) and group B to *M. suis* 'Guangdong' (AY492086). Tree was reprinted from HOELZLE *et al.*, 2010 ((128), pp. 129-133). The corresponding alignment can be found in the appendix on pp. 210-218.

Table V-7 Similarity matrix of the novel equine HM isolates to selected HM species

	Species	1	2	3	4	5	6	7	8	9	10
1	equine HM isolate 30/7 (FN421445)		97	98	94	94	90	87	83	83	82
2	equine HM isolate 32/2 (FN421443)	97		97	93	93	90	87	83	83	82
3	*C*M haemobovis' (EF460765)	98	97		94	94	88	86	80	82	79
4	*M. haemocanis* (AF197337)	94	93	94		99	88	86	80	79	80
5	*M. haemofelis* (U95297)	94	93	94	99		88	86	80	79	81
6	*C*M turicensis' (DQ825450)	90	90	88	88	88		90	82	82	83
7	*M. haemomuris* (U82963)	87	87	86	86	86	90		82	78	79
8	*M. wenyonii* (AY769937)	83	83	80	80	80	82	82		90	95
9	*M. suis* (AF029394)	83	83	82	79	79	82	78	90		90
10	*M. ovis* (AF338268)	82	82	79	80	81	83	79	95	90	

Similarity values were obtained from BLAST and are given in percent.

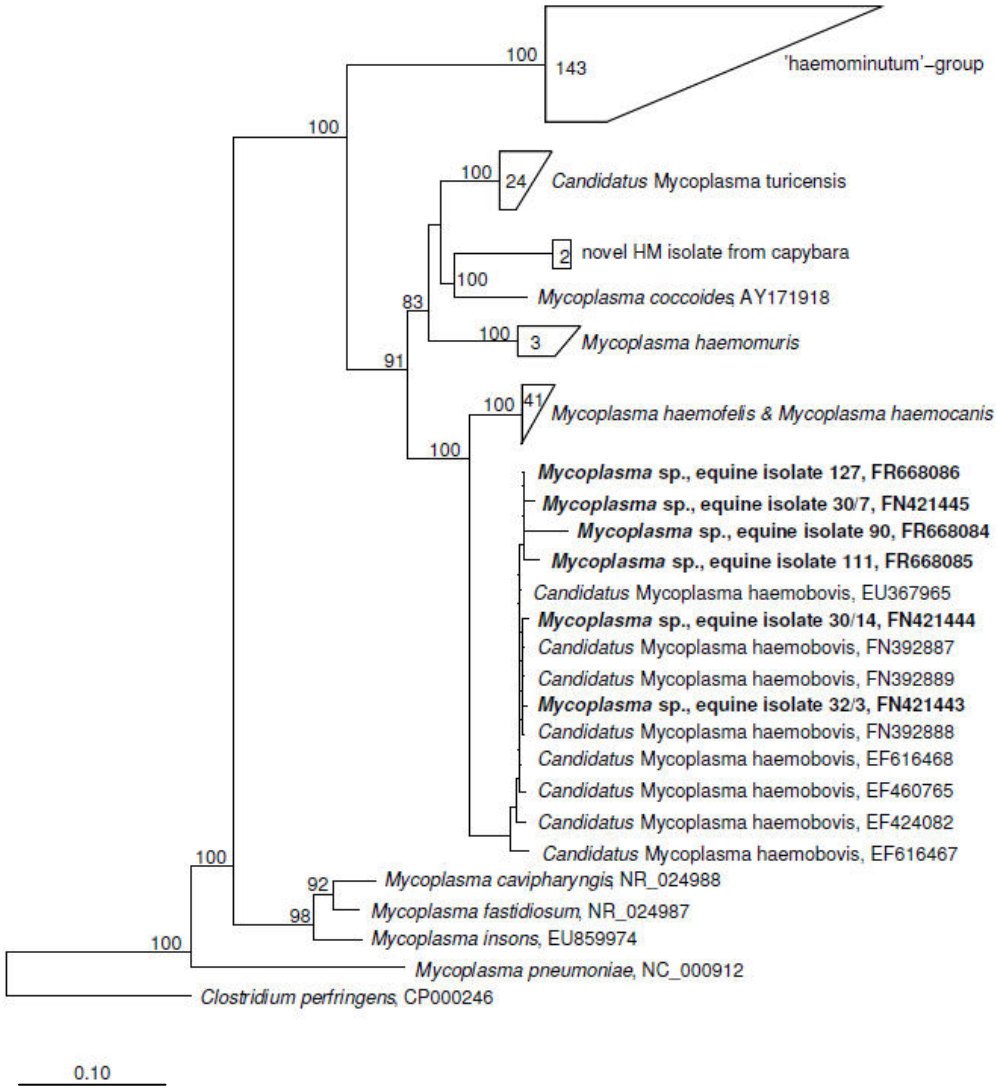

Figure V-5 Comparative sequence analysis using 16S rRNA sequences showing the position of the equine isolates among the HMs. Equine HM isolates are highlighted in bold-face. Size of sequences of isolate no. 30/7, 30/14 and 32/3 are between 880 and 916 bp, while sequences of isolate no. 90, 111, and 127 represent 117 bp-amplicons of the equine HM specific SYBR green I real-time PCR assay. The tree shown was produced by applying the maximum likelihood method in combination with a 25 % similarity filter. The numbers at the nodes indicate bootstrap values in percent (1000 bootstraps). Only values higher than 45 % are presented here. The scale bar represents the estimated evolutionary distance. *Clostridium perfringens* served as an outgroup for tree rooting. Clusters including more than two species were grouped. Size of triangle indicates homology within the clusters and the number inside of the triangle indicates the number of group members. An alignment comprising all equine HM and '*C*M haemobovis' isolates can be found in the appendix on pp. 207-209.

VI. DISCUSSION

VI. DISCUSSION

VI.1 Haemotrophic mycoplasmas in horses

In contrast to the well-known HM species in cattle, pigs, small ruminants, dogs, and cats (136, 210, 364), almost nothing was known about HM infections in horses with the exception of a 1978 case report based on cytology (101). The emerging significance of HM in domestic and free-ranging animals is evidenced by the rising number of newly described HM isolates and species in recent years (199, 263, 309, 314, 350, 351, 355, 360). Recently, putative HM infection in horses was discussed in online fora (e.g. *www.reitforum.de*, *www.hufreheforum.de*) and German journals ('*Pferdespiegel*', '*Pferdeheilkunde*' (93, 231)). NIEMENDAL analysed 108 blood smears for evidence of HM in his doctoral thesis and found a prevalence of 19.4-75.9 % (230, 231).

In the equine practice BEEKENHOF (Luneburg Heath, Northern Germany), a long history of purported HM infection in horses exists (DR. M. DIECKMANN, pers. comm.). Horses were reported to be meagre, do not develop well, showing a ruff haircoat, apathy, and reduced performance. Thorough clinical investigation and haematological analysis have revealed no abnormalities besides slight transient anaemia and, in some cases, a selenium deficiency. In peripheral blood smears stained with Giemsa, HM-like particles were observed (DR. M. WINKLER, synlab.vet, pers. comm.). Agents responsible for anaemia such as equine infectious anaemia virus, *Theileria* sp., *Babesia* sp., and *Ehrlichia* sp. were excluded.

Based on these reports, the present study was performed to evaluate the real clinical significance of equine HM infections. At the beginning, several DNA purification methods were tested for suitability of isolation of HM in horse blood. Since blood loads were very low in the affected horses examined by this study, bacterial DNA purification was a crucial step in sample processing. Unfortunately, every purification method results in partial loss of bacterial DNA, but most commercial kits are able to remove most PCR-inhibitory factors. However, successful detection of purified bacterial DNA with commercial kits requires at least 10^3 cells/mL blood (113, 379). Many of this study's horse blood samples had a HM blood load near the minimum required cell concentration. Efficiency of the phenol-chloroform extraction was higher, but the remaining phenol molecules interfered in both SYBR green I real-time PCR and conventional PCR approaches. Repeated EtOH precipitation removed phenol but led to DNA loss.

Although bacterial DNA was detected in 57 of 105 samples by conventional 16S rRNA PCR (primer pair hf1/ h1r), cloning and sequencing were only successful in three cases. Strangely, PCR results with different primer pairs did not match in all cases (Tab. V-4, pp. 69-73). These discrepancies may be explained by interference between non-specific DNA sequences and the desired bacterial DNA sequence at low bacterial blood loads in broad-spectrum PCR (379). ZUCOL *et al.* showed that detection of bloodstream infections necessitates a higher bacterial load than for detection of bacterial DNA in pure cultures (379). AKANE *et al.* demonstrated that haem in blood may inhibit amplification of DNA by PCR (4). Finally, remaining eukaryotic DNA of equine blood components may have interfered during PCR, inhibiting correct binding of oligonucleotides and Taq DNA polymerase to bacterial DNA. Thus, conventional PCR may not be the appropriate tool for detection of low-level HM infections, and conventional PCR results without confirmation by sequencing should be interpreted with caution.
Nevertheless, this Ph.D. thesis exhibits the first molecular proof that HM infections do occur in horses ((62); pp. 134-136).

After successful molecular proof of the existence of equine HM, a SYBR green I real-time PCR assay was developed and published ((63); pp. 141-165). This tool allows more sensitive, equine HM-specific detection in larger sample sizes compared to conventional PCR approaches. Recently, real-time PCR methods were developed for HM detection, leading to an increasing number of detection of novel HM isolates. These novel PCR methods have displaced hitherto used diagnostic tools based on microscopy; microscopy is too insensitive for detection of HM in animals with a subclinical course of disease or with low bacterial blood loads (16, 215, 269, 336). With the newly developed SYBR green I real-time PCR assay, an unambiguous and sensitive detection of HM in blood of affected horses is now possible. Equine HM prevalence was 33.2 % (70 of 211 samples exhibited a positive SYBR green I real-time PCR result; Tab. V-4, pp. 69-73). This value is high in comparison to the prevalence of HM in other animals: feline (0.5 to 8.5 % (361)) and canine HM in Switzerland (1.2 % (358)), Africa (6.0 % (11)), and Spain (0.6-14.3 % (271)), and *M. suis* in pigs in Germany (13.9 % (269)). Similarly high prevalences were observed for '*C*M haemobovis' in cattle in Japan (22.3 % (315)), '*C*M haemominutum' in cats in Australia and South Africa (24.0-38.0 % (362)), feline HM in Northern Italy (18.9 % (95)), and '*C*M haemolamae' in Switzerland (18.6 % (161)).

However, the HM prevalence in this study may represent an overestimate, as (i) most samples of this study were derived from a single breeding farm in Northern Germany, (ii) suspicious HM cases were previously reported at this farm (DR. M. DIECKMANN & DR. M. WINKLER, pers. comm.), and (iii) the sample number was rather low. In order to determine if the high HM infection prevalence is a regional phenomenon or a single-herd, sporadic event, the study should be repeated with a higher sampling number, and with samples originating from other locations, both in Germany and abroad. For example, *M. suis* was shown to occur throughout Germany (269), and feline HM cases have been reported from around the world (365). Varying prevalence data may reflect regional differences and occurrence of possible vectors.

Despite the development of highly sensitive real-time PCR assays, microscopy of peripheral blood smears stained with Giemsa or acridine orange is still the method of choice for detection of HM in many laboratories. Therefore, microscopic and PCR results were compared concerning sensitivity and specificity. PCR and microscopic results of Giemsa and acridine orange stained peripheral blood smears did not correlate well (Tab. V-3, p. 67). To a large extent, microscopically positive animals reacted negatively in PCR (9.2 % false-negative) and vice versa (38.8 % false-positive). False-negative microscopic detection may have resulted from low bacterial loads or inapparent infections with bacteria absent from blood. Blood loads of 4.4×10^6 '*C*M turicensis' copies/mL were reported to correspond to only one bacterium per 1000 to 10000 RBCs (215). This explains why a definitive microscopic detection of this feline HM species in blood has not met with success in the past few years (361, 367), even though, the 16S rRNA sequence of this agent was determined in 2005 (360). RITZMANN *et al.* reported that at least 10^6 *M. suis* cells/mL blood must be present for an unambiguous microscopic detection (269). Bacterial loads in the affected horses from this study were in the same low range (at an average of 1.67×10^7 cells/mL blood, range: 1.70×10^2 to 3.69×10^8 cells/mL), hindering the unambiguous identification of HM on the RBC surface using microscopy and conventional PCR methods. False-positive microscopic results may be due to confusion of HM with Howell-Jolly bodies, background debris, and staining artefacts (164, 198, 220, 314). Problems with false-positive and false-negative microscopic results are a well-known phenomenon in HM diagnostics (16, 118, 122, 269, 359). Evaluation of microscopic results depends on the observer's subjective impression, and there are no reference values, leading to difficulties when estimating the bacterial load in blood.

M. haemofelis can exhibit intense parasitaemia in the acute stage of infection, but bacteria may be rapidly cleared from blood, leading to negative cytological findings (5, 114). Because of this cyclic nature of parasitaemia, absence of bacteria from examined blood smears does not necessarily exclude an HM infection.

The false-positive and false-negative detection rates differed depending on whether Giemsa and acridine orange staining was used. Acridine orange staining is purportedly more sensitive than Giemsa staining (28). This study corroborated this earlier finding; the false-negative detection rate was lower for acridine orange stained blood smears (20.8 % and 60.5 %, respectively). However, the false-positive detection rate for acridine orange (34.2 %) was twice as high as samples stained with Giemsa (17.4 %). One possible explanation is that fluorescence staining may be more apt to form artefacts and staining precipitates, which may be later confused with bacteria.

Therefore, real-time PCR approaches should be used as routine diagnostic tools for HM detection since they are more sensitive than microscopy.

The probability of false-positive or false-negative SYBR green I real-time PCR results was low since specificity and sensitivity were checked by triplicate runs of serial dilutions of pCR30/7 plasmid DNA and testing of several mycoplasmal and non-mycoplasmal DNA (63). Due to the closed-tube system, the risk of carry-over contamination was low. However, it remains difficult to establish a novel diagnostic tool when a good reference method is missing. Longer storage of blood samples or DNA may have led to DNA degradation, and therefore, samples with a long history of storage may have appeared PCR negative by mistake. This may explain why several samples from 2008 reacted negative in the SYBR green I real-time PCR (performed in 2009/2010) even though, the samples were positive in 2008, as determined by microscopy and conventional PCR. DNA and blood had been stored at -20 °C, but DNA can degrade due to repeated freezing and thawing. Blood samples, which were sent by synlab.vet, had a long 'travel history'; they were shipped from the veterinarian to the lab, where they were further processed before being sent to Zurich. Taking this into account, the calculated blood loads in the samples with a longer storage or shipment history may have been too low or possibly PCR may have reacted negative in error.

In order to evaluate the clinical significance of the Winter 2009/2010 samples (no. 001-007, 010-012, 014, 015, 020-026, 029-031, 036, 048-142; n = 156; Tab. VIII-1, pp. 165-175), complete blood counts were prepared, and correlation of HM infection status and

haematological values was analysed. Blood counts of 24 horses, which were re-sampled one month later, and blood counts of horses without age information (n = 14) were excluded from this analysis. In 27 of 156 samples (17.3 %), for which a complete blood count was available, a normocytic, normochromic anaemia was observed. In twelve cases (7.7 %), a microcytic, hypochromic anaemia was found. In cattle infected with *M. wenyonii* and in sheep infected with *M. ovis*, both macrocytic, normochromic (172, 251) and normocytic, normochromic anaemia (307) were reported, while in cattle infected with '*C*M haemobovis', a hyperchromic, macrocytic anaemia was observed (315). However, equine HM infection was neither significantly associated with anaemia nor did bacterial blood loads correlate with haematological parameters (Fig. V-1, pp. 51-54; Fig. V-8, pp. 64-66). Absence of correlation between anaemia and HM infection was also reported for '*C*M haemolamae' (336) as well as for feline (16, 95, 361) and canine (236) HM, whereas *M. suis* infection in pigs is mostly accompanied by severe anaemia (normochromic, normocytic) along with strong correlation of haematocrit, haemoglobin, and RBCC (136, 269). In contrast, GUIMARAES *et al.* found no correlation for *M. suis* (107), and MUSEUX *et al.* reported a correlation between RBCC, haematocrit, and haemoglobin with blood loads of '*C*M turicensis' (215).

In all PCR positive animals independent of age, only RBCC and MCHC were significantly lowered. Also, a tendency of decreased eosinophil numbers was observed. Interestingly, in infected animals younger than one year-old, parameters indicative of anaemia (haematocrit, haemoglobin, and RBCC) were significantly lowered, but not in older infected horses. In the young animals, MCV and MCH were significantly increased (Fig. V-1, pp. 51-54). Thus, one can hypothesise that this type of infection runs a more severe course in younger horses, as it does in llamas (199, 263) and sea lions (351). *M. suis*- and *M. haemofelis*- parasitized RBCs may be sequestrated in the spleen. This may lead to the removal of the organisms by reticular cells without destroying the host cells (193, 194, 252). Similar removal mechanisms were reported for *Plasmodium falciparum* in malaria infected patients (277). Perhaps, similar effects are present in horses, hindering the detection of HM on the RBC surface.

In general, significant differences between the blood counts of horses younger than one year and horses older than one year were detected, independent of PCR status. Earlier reports showed that blood counts of young horses generally differ from older horses (47, 115, 341), but no distinct reference ranges for younger horses are given. Reference ranges can slightly vary from country to country and from race to race (especially for

drafting horses, thoroughbreds, and ponies (80, 115, 178, 341)). The horse's physical condition may also have an impact on haematological values (80).

In horses, HM infection seems to have a subclinical and asymptomatic course of disease, showing only low to moderate bacterial blood loads and low morbidity. For this reason, HM detection in horse blood and assignment of distinct clinical signs is complicated. Frequently, horses in this study only displayed unspecific clinical signs such as slimming, shaggy fur, apathy, and a poor performance. Therefore, bacterial loads near the detection limit do not seem to play an important clinical role. Similar clinical signs were reported for HM infection in llamas, where the infection has been called "failure to thrive syndrome" (263). In combination with "equine chronic fatigue syndrome", TARELLO found roundish particles on the RBC surface. The exact nature of these particles, however, was not analysed (318). In the review of RAZIN, also a connection of mycoplasmas and chronic fatigue syndrome was picked up (261).

Pathogenicity of HM species differ. *M. haemofelis* causes severe haemolytic anaemia in cats, while '*C*M haemominutum' and '*C*M turicensis' seem to be of lower morbidity (85, 359, 361). *M. haemofelis* may develop overt clinical signs in combination with severe anaemia in acutely infected cats, but chronically infected animals may not show clinical signs despite high HM blood loads (361). The bovine HM species *M. wenyonii* and '*C*M haemobovis' also differ in morbidity; HMs were detectable and blood count was changed without apparent or specific clinical signs in cattle (314, 315). NISHIZAWA *et al.* described low-level infections with poor PCR intensity in cattle showing no specific clinical signs (233). Even isolates of a single HM species may result in different pathogenicity, as shown by the novel *M. suis* isolate, which is able to invade RBCs. This new isolate leads to a fatal course of disease in pigs (106) while the classical isolate remains attached to the RBC surface. Infection with *M. haemocanis* is clinically inapparent, especially in the absence of immunocompromising conditions like splenectomy or coexistent infections (164, 236).

A subclinical course of infection may result in severe anaemia when the animal gets stressed or otherwise immunocompromised. Two cases of horses showing these unspecific clinical signs in combination with a progressive haemolytic anaemia (haematocrit of 0.26 L/L, decreasing to 0.13 L/L) were reported (DR. M. DIECKMANN, pers. comm.). HM-like particles could be detected in Giemsa stained blood smears (DR. M. WINKLER, pers. comm.). Disease in these horses had a fatal outcome, but, unfortunately, these ca-

ses happened before this study, and thus, the cases were not documented properly. More investigations in the field of clinical characterisation of this novel infection in horses must be done in future to establish a clear clinical picture and to examine the pathogenic potential of HM infection in horses. Presumably, affected horses' immune defence is reduced, and therefore the horse is more receptive for further infections. Furthermore, the impact of clinical manifestations and subclinical courses on breeding and sporting achievement of affected horses should be examined. In sows, for example, reduced fertility was reported (136). In the case of mild chronic courses of the disease, the owner has to count on a temporary reduction of use in sporting and breeding, and should expect retarded development in younger horses. In a more severe course of disease, even death may occur.

In some cases, two or three samples from one horse were collected over a time spanning from Spring 2008 to Winter 2009 (Tab. VIII-1, pp. 165-175). Seven of these horses exhibited a positive SYBR green I real-time PCR result in the samples from both years (Tab. V-4, pp. 69-73). Either they were infected again or they remained chronically infected. Chronic infection states, in which HM were only detected periodically and in low numbers in the blood, were also described for feline (361, 364) and canine HM (18, 164), *M. suis* in pigs (136), and '*C*M haemolamae' in alpacas (336). In horse no. 36, a "failure to thrive syndrome" was reported since birth (DR. M. DIECKMANN, pers. comm.). She was always a little bit smaller than her contemporaries and had a low nutritional condition of 3-4 of 10 (AAEP; 3 = thin, 4 = moderately thin). Accordingly, HMs were detected in blood smears in 2008 for the first time. HM infection was confirmed by SYBR green I real-time PCR in both samples from 2008 and 2009 (2.03×10^5 and 1.05×10^3 cells/mL blood). Ill-thrift and a "failure to thrive syndrome" were already reported for lambs (36, 53) and llamas (263). However, twelve horses that tested positive in the 2008 samples reacted negatively in the 2009 samples, and three horses that tested negative in the 2008 samples reacted positively in the 2009 samples. In the first case, the horses may have eliminated the infective agent from blood. In the second case, either the horses became infected during the months between the first and the second sampling, or the first sample was false-negative due to DNA degradation resulting from long storage.

In some blood samples, agglutination was observed. Agglutination was able to be resolved at 37 °C and re-induced at 4 °C. This incidental finding led to the hypothesis of occurrence of cold-reactive auto-antibodies. Unfortunately, Coomb's testing failed, since the test was not carried out immediately after sampling and RBCs started to lyse after some time of storage. Further studies should perform Coomb's testing directly after sampling for verification of the existence of cold agglutinins. However, rouleaux formation of equine RBCs and auto-agglutination may be mixed up (213), interfering with Coomb's testing. Thus, Coombs' testing may not work at all, and results should be interpreted cautiously. Cold agglutinins in conjunction with HM infection were observed in mice (48), cats (192, 328, 380), and dogs (17, 35). In *M. suis*-infected pigs, cold-reactive antibodies are a well-known phenomenon and contribute to clinical signs like acrocyanosis (136, 138). *M. pneumoniae* is capable of inducing cold agglutinins in humans (220). However, the pathogenic role of possible cold agglutinins in horses is unknown.

HM blood loads can be reduced by application of tetracyclines, as shown for several animals (e.g. pigs (136), cats (321), and llamas (336)). Tetracycline therapy in horses may be problematic, since they may be allergic to tetracycline drugs. Therefore, this kind of therapy should be reserved for the most severe courses of disease. In most cases, horses recovered by themselves after three to six months, helped by a vitamin-rich diet. In some cases, horses exhibited selenium deficiency, so food was supplemented with selenium (DR. M. DIECKMANN, pers. comm.). However, selenium concentration in serum was not serially analysed and a correlation of HM and selenium concentration is just speculation. It was shown that sheep with access to high quality food and/or trace element supplements can lessen the severity of anaemia (36, 110, 210).

Horses were analysed for risk factors for HM infection, like age, gender, or pregnancy, but no significant differences between PCR positive and PCR negative horses were found. This may be due to the small sample number, and therefore, the study should be repeated with a higher number of samples. For canine HM infections, housing in kennels, young age, crossbreeding, and mange infections are reported risk factors (164, 236). Increased susceptibility of kennel dogs may be due to infestations with *R. sanguineus*, "the kennel tick" (55), which is a known vector for *M. haemocanis* (283). Male

gender, old age, and feline leukaemia virus and feline immunodeficiency virus infections are risk factors for feline HM infections (16, 105, 322, 361, 362).
Immunosuppression and stress seem to play a role in the development of clinical signs. In winter, when horses are coming back from grazing land, the herds are often regrouped. Thus, horses may have stress due to conflicts of hierarchy and acclimatisation to a new environment. In this situation, the owners often report about horses which do not develop well, becoming meagre and apathetic. Development of overt 'haemobartonellosis' in llama is associated with stress conditions like shipping, relocation, parturition, and concurrent illness (199, 336). Most samples in this study were taken during this time period of regrouping the horses.

Since the infection often occurs in autumn after grazing season, transmission of HM is assumed to occur by vectors like ticks or horse flies. The grazing lands in the Weser marsh are highly infested with ticks (*Ixodes* sp.; DR. RAINER OEHME, *Landesgesundheitsamt Baden-Württemberg Ref. 93, Allgemeine Hygiene und Infektionsschutz, Stuttgart, Deutschland*, pers. comm.). Seasonal correlation of HM infection in cats (95) and pigs (173) supported the assumption of transmission via blood-sucking vectors. The seasonal aspect is also known for further tick-borne diseases (181, 200), which is in accordance with seasonal differences in tick abundance (280). Transmission via blood-sucking arthropods was shown for several HM species including ticks, lice, flies, and mosquitoes (22, 139, 177, 283, 284). In contrast, canine or feline HM was not detected in unfed ticks (*Ixodes* sp.) in Switzerland (363), which may offer an explanation for the low prevalences of canine and feline HM in Switzerland. Perhaps, relevant vector insects are not indigenous in Central Europe (3) but rather in southern countries (37, 61, 196, 267, 335). In southern regions of Europe, where *R. sanguineus* is indigenous (23, 96), higher prevalences of canine HM were observed (11, 164, 165, 236, 271). Higher prevalences of feline HM were found in South and West Switzerland, which are known for higher mean annual temperatures, and where potential vectors *R. sanguineus* and *D. reticulatus* are indigenous (3, 23, 361). Furthermore, vector transmission was substantiated by NASH & BOBADE, who found a higher prevalence of *M. haemofelis* in cats infested with fleas (216). ROURA *et al.* reported that dogs infected with vector-borne infections (e.g. *Leishmania infantum*, *Ehrlichia canis*, *Anaplasma platys*, *Babesia canis*) showed a higher prevalence of HM (271).

The idea of transmission of equine HM via ticks can be supported on several grounds: (i) horses were often infested with high numbers of ticks (DR. MICHAEL DIECKMANN, pers. comm.), and (ii) in the region of Weser marsh (grazing lands in summer), *Ixodes* sp. are widely distributed (280). *I. ricinus* as well as *D. reticulatus* and *D. marginatus* are known to transmit vector-borne pathogens like *Babesia* sp., *Theileria* sp., *Ehrlichia* sp., *Borrelia burgdorferi*, and feline HM (15, 180, 273, 280, 319, 363). Due to climate change (humid summers and mild winters), ticks have become more widely distributed in increasing numbers in northern regions (181, 200), leading to higher prevalences of previously rare diseases (e.g. babesiosis, anaplasmosis, ehrlichiosis) (56, 117). While *I. ricinus* is widely distributed in Germany (200), *D. reticulates*, *D. marginatus*, and *Haemaphysalis* sp. were only found sporadically (14, 15, 180). These species were previously restricted to Southern or Eastern Germany (180, 203), but are now starting to appear in Northern Germany (117). One has to assume that *Dermacentor* sp. will be on the rise (DR. RAINER OEHME, pers. comm.), especially in meadow- and marshlands (117). *Ixodes* sp. and *Dermacentor* sp. are known to feed on small and large mammalian animals; *Ixodes* sp. prefer rodents (280), and *Dermacentor* sp. infest mainly horses and dogs (117). Wild animals like deer and boar may be highly infested (117, 280). Therefore, a systematic study of tick distribution in Northern Germany should be performed, and unfed ticks and ticks collected from animals should be tested for HM. The SYBR green I real-time PCR assay developed in this thesis work would provide a useful instrument for performing these studies. The prevalence of tick-borne diseases (equine babesiosis, equine theileriosis) is significantly lower in Germany and Switzerland than in France, Spain, and Portugal (286). However, infection rates and number of ticks harbouring bacteria and viruses are increasing (117, 181, 200).

In utero transmission was shown for a four day-old cria and an one day-old llama (6, 81). Also, transplacental infection of *M. haemocanis* in dogs (171) and *M. haemomuris* in rodents may occur (240). Interestingly, two mares (no. 49 and 15) and their corresponding offsprings (no. 67, born in 2009, and no. 85, born in 2007) were PCR positive. Unfortunately, no data was available on the health status of the foals before blood sampling in November 2009. Thus, transplacental transmission could occur in horses as well and should be taken into account in future studies.

Researchers assume that only a small fraction of bacteria is known thus far; especially uncultivable bacteria are hard to detect. Design of fluorescently labelled nucleic acid

probes based on comparative sequence analysis provides a powerful tool for identification of novel, uncultivable bacterial species (8, 59, 303). Due to the integration of a signal amplification step, sensitivity of CARD-FISH (catalysed reporter deposition fluorescence *in situ* hybridisation) is ten to twenty times higher than the conventional FISH approach. CARD-FISH also allows detection of cells with low numbers of ribosomes and individuals against highly auto-fluorescent backgrounds (8). Since at least 10^3 cells/cm^2 should be present, which corresponds to approximately 10^5 cells/mL blood (8), detection of low-level HM infections may be difficult. However, HM detection was possible in one horse sample with a blood load of 7.70×10^5 cells/mL blood using an universal bacterial probe targeting rRNA (EUB338; Fig. V-8, p. 68). Development of a probe specific to the novel equine isolate would allow accurate and sensitive detection of HM in blood. However, this specific CARD-FISH assay remains to be established. To the author's knowledge, the only report of HM detection via FISH was very recently published for feline HM (244): *M. haemofelis* was detected by FISH in blood and tissues of a cat exhibiting high blood loads, but detection of '*C*M turicensis' and '*C*M haemominutum' in tissues failed – presumably because of low blood loads (244).

To further characterise the novel equine HM isolate, scanning electron microscopy was performed (Fig. V-5, p. 59). Rarely, round particles of about 300 nm in diameter were found on the RBC surface, but more frequently in between RBCs. The size of this novel infective agent was near the 250 nm detection limit in light microscopy, giving rise to another possible explanation for the low sensitivity of light microscopic detection methods. The observed morphology and cell size were in accordance with those found in other HM species, e.g. *M. haemomuris* (300-750 nm (316)), *M. haemofelis* (500 nm (60, 152, 367)), *M. ovis* (0.5-1 µm (223)), *M. suis* (up to 1 µm (106)), '*C*M haemolamae' (400-600 nm (263)), '*C*M haemominutum' (300 nm (86, 367)), and '*C*M turicensis' (300 nm (367)). This substantiates the suspicion that the herein reported eperythrocytic structures indeed represent HM in equine blood. However, pleomorphic forms (e.g. ring- or rod-shaped cells, and cell chains) as described for other HM species (376), were not detected. Bacteria were often detected lying in small grooves. Appearance of invaginations and indentations was also described for *M. wenyonii* (31), *M. ovis* (109), and *M. suis* (376).

Mostly, blood was anti-coagulated by EDTA, for which a detachment effect was reported (5, 265). Later, SEM samples were anti-coagulated with citrate. Unfortunately,

detachment effects still appeared. This may be due to long delays between sampling and fixation or other unknown causes in sample preparation.

'*Haemobartonella*'-like species are only rarely found free in the plasma, in contrast to the more freely detected '*Eperythrozoon*'-like species (220). For *M. coccoides*, a loose contact between parasite and host cell, where the parasite did not lie in an indentations alongside free cells in the plasma, was observed (316). *M. coccoides* was reported to have low pathogenicity; the novel equine HM species also seem to be of low pathogenicity. *M. wenyonii* – a bovine HM of low pathogenicity (172, 315) – does not induce significant membrane alterations and forms only slight depressions (163). *M. suis* (106, 376) and *M. haemofelis* (152), on the other hand, form deep indentations in the RBC membrane and cause severe disease in pigs (136) and cats (210). Thus one may postulate that the intensity of adherence and membrane alterations influences the pathogenicity of HM. Structures resembling attachment and invagination scars (106) were detected on the surface of equine RBCs. Possible attachment scars were also found for feline RBCs infected with '*C*M turicensis' (367) or *M. haemofelis* (152). These lesions may hypothetically result in increased osmotic RBC fragility (191, 374); increased osmotic RBC fragility was shown for feline HM (151, 360) and was also detected in equine RBCs in this study. ZACHARY & BASGALL found that membrane alterations of porcine RBCs retained, although *M. suis* cells were cleared from blood, implicating a participation of RBC-own cytoskeleton structures in irreversible RBC deformation (376). This hypothesis was supported by findings of our institute; *M. suis* binds to RBCs' actin (78). In contrast, llama RBCs seem to be more resistant against membrane deformations; RBC deformations in HM infected llamas were not found (166, 263). Membrane deformations and indentations are a common feature of other blood-borne infections, such as *Bartonella bacilliformis* in humans (19). *B. bacilliformis* secrets a protein that causes membrane deformation and aids in binding and invading the RBCs (204). *Mycoplasma* infections may actively induce eryptosis in the cases of *M. hyorhinis* (241) and *M. suis* (79).

More often than single HM, microcolonies were detected on the surface of RBCs and in between RBCs, 'sticking' them together. At first glance, these structures looked like 'dirt'. For pigs naturally infected with *M. suis*, similar structures were observed (DR. KATRIN GROEBEL, IVB, pers. comm.). Microcolonies were also detected in *M. suis in vitro* cultures established by SABRINA SCHREINER (Ph.D. Thesis, IVB), and SEM of *M. pneumoniae* have revealed similar cauliflower-shaped microcolonies on human

RBCs (257). Unfortunately, due to preparation instability, it was not possible to take a closer look on microcolony structures in equine samples. Confirmation by immunogold staining methods should be done in future. Results from experimental infection studies are often obtained from splenectomised or otherwise immunocompromised animals, and data from experimentally infected animals cannot be mapped one-to-one to the naturally infected animals presented in this study. Experimental infection studies leave out important interactions between the bacteria and the host immune system. Further studies on natural HM infections in animals have to show the implications of possible microcolonies in blood of affected animals. To the best of the author's knowledge, microcolonies have not yet been detected in experimentally infected animals, and these structures are only known from naturally infected animals. Perhaps, the ability to 'hide' behind fibril structures in microcolonies helps the bacteria to evade the host immune system.

In order to analyse the fine structure of the novel equine HM species and to further examine possible intracellularity, transmission electron micrographs have to be prepared in future investigations. Intracellularity may preclude detection of HM by light microscopy and SEM in late stages of infection. In experimentally *M. suis*-infected pigs on day eleven post infection, for example, bacteria were not or only rarely detected on the RBC surface despite the high blood loads of 10^{10} to 10^{11} cells/mL blood (106). *M. suis* was the first HM species to be found inside of RBCs. Other blood-borne pathogens that were previously considered to be purely epicellular were also recently found inside RBCs (e.g. *Bartonella henselae* (170)).

VI.2 Phylogenetic analysis of novel HM isolates

The *Mycoplasma* phylogeny published in this thesis is in good agreement with previously published studies (155, 208, 219, 220, 265): The haemotrophic mycoplasmas clearly belong to the large group of *Mollicutes*, formed a separate cluster within in the group of mycoplasmas, and they are closest related to members of the 'pneumoniae-group' (Fig. V-9, p. 77). Within the HM group, two distinct subclusters are present. Analysis of secondary structure of 16S rRNA substantiated these findings of two separate clusters. Four different structural features were found (Fig. V-10 & Fig. V-11, pp. 78-79). The variability of certain loops was reported by WOESE (369). PETERS *et al.* introduced the designations 'haemominutum-group' and 'haemofelis-group' (243) for the two HM subclusters. JOHANSSON *et al.* (155), NEIMARK *et al.* (220), and MESSICK *et al.* (208) reported a characteristic truncation (*E. coli* numbering: 453-481), which is present in the 'haemofelis-group' but absent in the 'haemominutum-group' (Tab. V-5, pp. 74-76). The well-established species *M. suis*, *M. wenyonii*, and *M. ovis* (formerly known as *Eperythrozoon* sp.) belong to the 'haemominutum-group', while the 'haemofelis-group' comprises the long-known species *M. haemofelis*, *M. haemocanis*, and *M. haemomuris* (formerly classified as *Haemobartonella* sp.). The two genera *Eperythrozoon* and *Haemobartonella* were previously separated due to morphological differences (220). Due to phylogenetic analyses presenting two distinct clusters, one may suspect that this differentiation of the pre-sequencing era was justified. The only exception to this finding is *M. coccoides*, which belongs to the 'haemofelis-group', but was formerly known as *E. coccoides*.

All calculated trees exhibited similar global tree topologies. However, low bootstrap values indicated unstable local branching orders. Branching order within the clusters varied between neighbour joining and maximum likelihood trees. When interpreting trees, one must be aware that "any phylogenetic tree represents only a possible model of the process and results of evolution" (188), since "different treeing methods are based on different models of evolution" (186), and that "trees are not stable structures but dynamically change in response to acquisition and inclusion of new data" (188). Nevertheless, assignment of the HM species to the two subclusters was in accordance in all treeing methods. Within the 'haemominutum'-group, close relationships between cervid HM species and *M. wenyonii*, as well as closest relationship between '*C*M haemozalophi' and '*C*M haemolamae' were displayed in all treeing methods, as reflected by high bootstrap values. Also, '*C*M haemominutum' and '*C*M haematoparvum' exhibited close

relationships in all calculated trees. However, branching order of '*C*M erythrodidelphis' and '*C*M kahanei' varied. Within the 'haemofelis-group', the close relationship between *M. haemofelis*, *M. haemocanis*, '*C*M haemobovis', and the novel equine HM isolate was confirmed in all calculated 16S rRNA trees by high bootstrap values. On the other hand, branching order within the subcluster comprising *M. haemomuris*, *M. coccoides*, and '*C*M turicensis' was unstable using different treeing methods in combination with different similarity filters.

Some species are closely related, displaying only minor differences in the 16S rRNA sequence. *M. haemofelis* and *M. haemocanis* show 97-98 % 16S rRNA sequence identity but differ more in *rnpB* sequences (26, 243). Infection studies revealed no cross-infectivity (84), and therefore, designation as separate species seems to be justified. Thus, 16S rRNA sequences may not be the sole appropriate tool for delineation of closely related HM species. Species definition is somewhat arbitrary in bacteriology, and the concept of genospecies was introduced to define members of one species as having "70 % or greater DNA-DNA-relatedness with 5 °C or less ΔT_M" (8, 349, 356). This corresponds to an approximate 16S rRNA identity of 97-98 %. Although the elaborate and time-consuming DNA-DNA hybridisation technique is regarded as the "gold standard" for species delineation (356), 16S rRNA sequence analysis is widely used for classification and phylogenetic purposes (302). However, in cases of closely related organisms, 16S rRNA's resolution is not sufficient (88, 302). As was stated previously, 16S rRNA may not be the appropriate tool for phylogenetic classification of mycoplasmas, since they "tend to lack a higher than normal fraction of the highly conserved oligonucleotide sequences found in almost all 16S rRNA catalogues from normal eubacteria" (368), potentially resulting in "inexact or incorrect phylogenetic placement" (368). Housekeeping genes like RNase P RNA subunit (*rnpB*), elongation factor Tu (EF-Tu), or the β-subunit of RNA polymerase (*rpoB*) may provide more appropriate molecules to differentiate mycoplasmas (26, 159, 168, 243), as was shown for other closely related bacteria (e.g. *Chlamydia* sp. (123), *Streptococcus* sp. (71, 317), *Staphylococcus* sp. (70), and *Enterobacteriaceae* (211)). Some *Bacillus* strains share more than 99 % 16S rRNA sequence identity but show less than 70 % DNA-DNA-hybridisation relatedness (88, 349).

In the case of *M. ovis* and '*C*M haemovis', differentiation between these two species based on 16S rRNA sequencing is not justified in the author's opinion, as they both infect sheep and differ only in 17 bp (Fig. V-12, Tab. V-6, p. 80). Accordingly, no distinct branching order of these isolates was observed. Thus, these two isolates may represent

strain variations of the same species. However, this has to be confirmed by further phylogenetic analyses.

Phylogenetic analysis of the novel equine HM was complicated by the low bacterial blood loads which inhibited conventional PCR approaches. This is a well-known phenomenon in bloodstream infections (329). In three cases, sequencing of approx. 900 bp was successful (acc. no. FN421443, FN421444, FN421445). However, definite phylogenetic classification is hampered, when only part of the 16S rRNA gene is known (302, 349), especially if the more variable 5'-region is missing (186). The complete 16S rRNA gene has a size of about 1400 bp (*M. suis*: 1469 bp (238), *M. haemofelis*: 1429 bp (12)). *Rhizobium galegae*'s phylogenetic position differs based on use of partial or complete 16S rRNA sequences (349). For definite classification of the novel equine HM isolate, at least the complete 16S rRNA sequence is required. Ideally, additional phylogenetic marker genes should be analysed, and DNA-DNA hybridisation experiments should be performed.

Interestingly, infections of cattle herds with '*C*M haemobovis' were reported in the same region (130) as the novel equine HM isolate. Since these two isolates showed minor differences in 16S rRNA sequences (97-98 % sequence identity), their relationship situation seems to be quite similar to that of *M. haemofelis* and *M. haemocanis*. Further phylogenetic and cross-infectivity analyses are necessary to determine if the novel equine HM isolate is in fact a separate species. Therefore, the designation '*Candidatus* Mycoplasma equi' would be proposed, as the '*Candidatus*' designation is reserved to describe the provisional status of new, incompletely described taxa (214, 220). Unfortunately, amplification of the complete 16S rRNA gene as well as of the *rnpB* gene of equine HM isolates failed, since the low bacterial blood loads complicate conventional PCR approaches and cloning. Additionally, since the agent was not cultivable, it was not possible to extract the high amounts of bacterial DNA required for DNA-DNA hybridisation experiments.

There are, however, examples of closely related *Mycoplasma* species which only exhibit slight differences in 16S rRNA sequences; species delineation via DNA-DNA hybridisation and serological data confirms their classification of different species (248). If further analyses reveal no distinct species delineation of the novel equine HM isolate, then '*C*M haemobovis' can probably cross-infect cattle and horses. *Mycoplasma* host specificity has been questioned recently (249). *M. ovis*, for example, can infect a broader host range, including sheep, goat and deer (51, 52, 220). In that case, a pathovar or subspe-

cies designation in mycoplasmology should be considered. For historical reasons, HM species were named after the animal species they were isolated from, as clear phylogenetic analyses were not possible in the past. This convention in nomenclature may lead to classification of the same organisms as different species.

'*C*M turicensis' clustered together with rodent HM species (*M. haemomuris* and *M. coccoides*). Thus, the corresponding HM species may have been developed in co-evolution with their hosts, or HM may have been transmitted from prey to predator or vice versa and later adapted to the new host. However, rodents probably do not play an important role as reservoir (363).

VI.3 Conclusion

In this thesis work, the first molecular proof of the existence of a HM infection in horses was presented, and a SYBR green I real-time PCR assay specific to the novel equine HM isolate was established. Furthermore, the significance of equine HM infections was analysed; this kind of infection seems to have a more severe course of disease in young animals. In the last decade, new HM species were regularly discovered. Advanced diagnostic techniques, such as PCR, real-time PCR, and FISH, simplified the detection of uncultivable bacteria and contributed to an increasing number of new discoveries. New sequencing technologies facilitated their recognition and classification. The herein published *Mycoplasma* phylogeny was in good accordance with previous publications. Due to climate change, possible vectors have opened up new methods of distribution, and therefore, vector-borne infections are on the rise. With the emergence of immunocompromising diseases, organ transplants, and cancer therapies, HM's zoonotic potential deserves careful future study, as HMs were recently isolated from immunocompromised humans (67, 72).

VII. LITERATURE

VII. LITERATURE

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VIII. APPENDIX

VIII. APPENDIX

VIII.1 Publications

On the following pages publications prepared during this study will be presented in chronological order.

1. HOELZLE, K., M. ENGELS, M. M. KRAMER, M. M. WITTENBRINK, **S. M. DIECKMANN** & L. E. HOELZLE. 2010. Occurrence of *Mycoplasma suis* in wild boars (*Sus scrofa* L.). Vet Microbiol 143:405-409 pp. 129-133
2. **DIECKMANN, S. M.**, M. WINKLER, K. GROEBEL, M. P. DIECKMANN, R. HOFMANN-LEHMANN, K. HOELZLE, M. M. WITTENBRINK & L. E. HOELZLE. 2010. Haemotrophic *Mycoplasma* infection in horses. Vet Microbiol 145:351-353 pp. 134-136
3. HOELZLE, K., M. WINKLER, M. M. KRAMER, M. M. WITTENBRINK, **S. M. DIECKMANN** & L. E: HOELZLE. 2011. Detection of *Candidatus* Mycoplasma haemobos in cattle with anaemia. Vet J 187:408-410 pp. 137-139
4. **DIECKMANN, S. M.**, K. HOELZLE, M. DIECKMANN, I. STRAUBE, R. HOFMANN-LEHMANN, & L. E. HOELZLE. Significance of hemotrophic mycoplasmas in horses: A disease of young animals? 2011. submitted to J Clin Microbiol. Under review. pp. 140-164

VIII.1.1 HOELZLE *et al.* (2010): Occurrence of *Mycoplasma suis* in wild boars (*Sus scrofa* L.)

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Short communication

Occurrence of *Mycoplasma suis* in wild boars (*Sus scrofa* L.)

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ABSTRACT

Porcine infectious anemia is a well-known disease that occurs worldwide and is caused by the uncultivable hemotrophic bacterium *Mycoplasma suis*. In this study the occurrence of *M. suis* in wild boars was investigated by employing a quantitative real-time LightCycler PCR. *M. suis* infections were detected in 36 out of 359 wild boars (10.03%). Sequencing of the 16S rRNA gene and subsequent phylogenetic analysis revealed the existence of two genetically distinct *M. suis* subtypes in the wild boar population: one subtype was >99.0% identical to known American and European *M. suis* isolates, and the second subtype showed the highest homology to known Chinese isolates. In summary, this is the first detection of *M. suis* in wild boars. The role of *M. suis* as pathogen in wild boars has yet to be established, but the present findings revealed a possible wildlife reservoir for these bacteria.

1. Introduction

Mycoplasma suis belongs to a group of highly specialized uncultivable hemotrophic bacteria which parasitize erythrocytes of several animal species (Messick, 2004; Hoelzle, 2008). *M. suis* causes infectious anemia in pigs being a globally widespread disease that causes major economic losses in swine production. The acute disease is accompanied by severe bacteremia and sometimes death of young piglets and pregnant sows (immediately prepartum) as well as loss of fattening pigs under stress (Zachary and Basgall, 1985; Messick, 2004). In chronically infected pigs bacteremia is low and the clinical signs vary ranging from mild icteroanemia, general unthriftiness, poor growth rates or bad reproductive performance to increased susceptibility for other infectious diseases (Henry, 1979; Brownback, 1981; Zinn et al., 1983; Schweighardt et al., 1986). The role of *M. suis* as a pathogen in pigs has long been established: *M. suis* was first described in the United States in 1932 (Kinsley, 1932). Furthermore, *M. suis* infections in pig husbandry have continuously been reported worldwide over the past 75 years (Dipeolu et al., 1982; Schuller et al., 1990; Hoelzle et al., 2003, 2007b; Messick, 2004; Wu et al., 2006; Guimaraes et al., 2007; Ritzmann et al., 2009). Current studies determined prevalences of 13.9% in Germany (Ritzmann et al., 2009) and 18.2% in Brazil (Guimaraes et al., 2007).

Wild boars are known reservoirs for porcine pathogens such as brucellae (Gibbs, 1997), classical swine fever (Ruiz-Fons et al., 2008), and trichinellae (Van der Giessen et al., 2001) that may be transmitted to domestic animals by contact. However, for *M. suis* no data is available on the distribution of these bacteria in the wild boar population. This lack of data is mainly due to the general problems in diagnosing *M. suis*, i.e. the lack of cultivation systems. The introduction of PCR detection methods as of the 1990 (Gwaltney et al., 1993; Messick et al., 1999; Hoelzle et al., 2003) and the advancement of PCR methods, i.e. quantitative PCR assay (Hoelzle et al., 2007a) have greatly improved the possibilities to diagnose *M. suis* infections.

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Table 1
Sample origin, prevalence, and LC PCR results.

Origin	No. of samples	No. of PCR-positive samples	Mean bacterial load (copy/ml blood)
Hunting ground A	13	1 (7.69%)	9.74×10^3
Hunting ground B	15	1 (6.66%)	6.58×10^4
Hunting ground C	10	0	–
Hunting ground D	14	0	–
Hunting ground E	23	0	–
Hunting ground F	19	1 (5.26%)	1.38×10^3
Hunting ground G	30	0	–
Hunting ground H	3	0	–
Hunting ground I	38	2 (5.26%)	3.32×10^4
Hunting ground J	20	0	–
Wild boar enclosure	174	31 (17.82%)	9.50×10^4

The present study was aimed at investigating the occurrence and prevalence of *M. suis* in wild boars. Furthermore, we focused on characterizing molecularly the identified *M. suis* isolates.

2. Materials and methods

2.1. Sample collection

The study involved collecting a total of 359 EDTA-anti-coagulated blood samples from wild boars which were hunted down across a period of two months (December 2007 and January 2008). The animals originated from ten hunting grounds and one wild boar enclosure in Southwest Germany (Table 1). Among the animals 167 were adult females and 192 adult males.

2.2. DNA extraction

200 μl volumes of whole anti-coagulated blood were mixed with 200 μl lysis buffer (10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 320 mM sucrose, 1% (v/v) Triton X-100) and centrifuged (8.000 × *g*, 22 °C, 60 s). The pellet was suspended in 400 μl lysis buffer and then centrifuged again. Whole DNA was extracted by means of the GenElute Bacterial Genomic DNA Kit (Sigma, Buchs, Switzerland).

2.3. Quantitative M. suis-specific real-time PCR

M. suis DNA was detected and quantified with the LightCycler 2.0 System (Roche Diagnostics, Rotkreuz, Switzerland), as described previously (Hoelzle et al., 2007a).

2.4. DNA sequencing, data analysis and phylogenetic tree construction

PCR reactions were performed using the 16S rDNA universal oligonucleotides: 16S_27F CAGAGTTTGATGCTGCTGGCTCAG and 16S_1492R TACGGYTACGTTACGACT. PCR was performed by using the HotStarTaq Polymerase Master Mix (Qiagen, Hombrechtikon, Switzerland), 0.5 μM of each primer, and an annealing temperature of 55 °C. Amplicons were purified with the QIAquick PCR Extraction Kit (Qiagen) and sequenced (MWG Biotech, Martinsried, Germany). Nucleotide sequences were analyzed by using the FASTA algorithm (Biocomputing service, University Zurich, www.bio.unizh.ch/). Revealed sequences were submitted to GenBank (accession nos. FN391018, FN391019; FN391020; FN436019; FN436018; FN436017; FN436016; FN436015; FN436014; FN436013; FN436012; FN436011; FN436010; FN436009).

Data (prevalence, *M. suis* blood loads, geographical origin, gender) were compiled and analyzed using Excel for Windows (Microsoft, Wallisellen, Switzerland), Analyze-it (standard edition Software ONE AG), and Origin (Redacom) software. 95% confidence intervals (CI) were calculated for PCR results.

For the phylogenetic analysis *Mycoplasma* 16S rRNA gene sequences were obtained from GenBank. Phylogenetic analyses were carried out using the ARB software package (Ludwig et al., 2004). Sequence alignment was performed using the ClustalW tool (ARB software package). A phylogenetic tree was constructed by using the neighbor-joining method in combination with the Felsenstein correction model. A minimal-similarity filter was used, which retained only positions conserved in at least 25% of the sequences selected. The data set was re-sampled 1000 times by bootstrapping.

3. Results

3.1. Prevalence of M. suis in wild boars

M. suis DNA was detected in 36 out of 359 wild boars (10.03%; 95% CI: 6.92–13.14%). Most of the PCR-positive animals originated from the one wild boar enclosure ($n = 31$), the remaining five PCR-positive animals originated from four different hunting grounds. Quantification of the bacterial loads revealed a mean value of 8.60×10^4 *M. suis* copies per ml blood (95% CI: 1.65×10^5 to 2.63×10^5; range: minimum load 2.86×10^2; maximum load 1.11×10^6; Table 2). No significant correlation between PCR results and the gender of the wild boars was found.

3.2. Molecular characterization of M. suis from wild boars

To further characterize the *M. suis* isolates obtained from the blood of wild boars at the molecular level 18 isolates were chosen randomly. Based on the 16S rDNA sequences the wild boar isolates could be differentiated into two groups: group A consisted of twelve isolates

Table 2
M suis positive wild boars, origin, LC PCR results and 16S rDNA grouping.

Sample designation	Gender	Sample origin	*M. suis* load/ml blood	16S rRNA gene group[c]
029	F[a]	Wild boar enclosure	9.28×10^4	n.d.[d]
031	F	Wild boar enclosure	3.21×10^4	n.d
037	F	Wild boar enclosure	2.87×10^4	B
039	F	Wild boar enclosure	2.87×10^4	A
041	F	Wild boar enclosure	9.77×10^3	B
047	F	Wild boar enclosure	4.74×10^2	n.d
051	M[b]	Wild boar enclosure	6.06×10^5	A
065	M	Wild boar enclosure	2.76×10^4	A
073	F	Wild boar enclosure	3.97×10^2	n.d
074	F	Wild boar enclosure	1.24×10^5	B
083	M	Wild boar enclosure	1.75×10^5	n.d
093	F	Wild boar enclosure	1.26×10^5	A
128	F	Wild boar enclosure	1.38×10^3	A
146	M	Wild boar enclosure	3.31×10^2	n.d
159	M	Wild boar enclosure	9.93×10^3	n.d
169	M	Wild boar enclosure	3.27×10^4	A
170	M	Wild boar enclosure	9.71×10^3	n.d
176	M	Wild boar enclosure	1.03×10^4	A
178	F	Wild boar enclosure	1.22×10^5	B
180	M	Wild boar enclosure	2.09×10^5	n.d
182	M	Wild boar enclosure	2.88×10^3	n.d
183	F	Wild boar enclosure	8.51×10^3	B
215	F	Wild boar enclosure	1.11×10^6	A
216	F	Wild boar enclosure	5.24×10^4	n.d
218	F	Wild boar enclosure	1.75×10^4	A
219	F	Wild boar enclosure	4.61×10^4	n.d
221	M	Wild boar enclosure	6.41×10^4	n.d
222	M	Wild boar enclosure	8.14×10^3	n.d
223	M	Wild boar enclosure	2.22×10^3	n.d
226	M	Wild boar enclosure	4.25×10^4	A
227	M	Wild boar enclosure	2.96×10^4	A
228	M	Hunting ground	9.98×10^3	B
266	F	Hunting ground	6.58×10^4	A
313	M	Hunting ground	1.66×10^4	n.d
335	F	Hunting ground	4.98×10^4	n.d
350	F	Hunting ground	9.74×10^3	n.d

[a] Female.
[b] Male.
[c] Grouping according to the 16S rDNA sequence analysis.
[d] Not done.

(Table 2). Group A 16S rDNA sequences were 100% identical to each other. Furthermore, group A sequences revealed ≥99.00% identity with 16S rDNA of the *M. suis* strain Illinois (accession no. U88565) and with 16S rDNA of isolates found in pigs in Germany (accession no. FN391021, FN391022). Group B consisted of six isolates (Table 2). The group B sequences were also 100% identical to each other. However, group B 16S rDNA sequences revealed only 96.92% identity to those of group A isolates. When comparing to GenBank entries group B 16S rDNA sequences showed 99.60% identity to one isolate found in China (*M. suis* Guangdong; accession no. AY492086). Based on the 16S rDNA sequences a phylogenetic tree was constructed (Fig. 1). The calculated tree showed one single cluster for the porcine hemotrophic mycoplasma sequences. The 16S rDNA sequences obtained from the wild boars clustered with those of other porcine hemotrophic mycoplasmas. Within the cluster of porcine hemotrophic mycoplasmas two sub-clusters could be distinguished: the group A wild boar isolates sub-cluster with the *M. suis* strains Illinois, the group B wild boar isolates sub-cluster with the Chinese *M. suis* isolate Guandong.

4. Discussion

M. suis infections are an economic problem for the pig industry throughout the world (Messick, 2004; Hoelzle, 2008). To our knowledge this is the first study dealing with *M. suis* infections in wild boars. The animals used here originated from ten hunting grounds and from one wild boar enclosure. When analyzing the samples we found *M. suis* in 10.03% of the animals. However, this value does not reflect the real prevalence since hunting grounds and the wild boar enclosure (i.e. ground with fence) probably represent two completely distinct epidemiological situations: in the first, contacts between animals are determined by natural behavior. In the second, the fences and the higher population density lead to frequent and close contact between wild boars causing a higher infection rate. Furthermore, pot-bellied pigs were held in the area of the investigated wild boar enclosure which might also play an important epidemiological role. Within a pig population *M. suis* can be transmitted by direct contact, e.g. by contact between piglets and sows or during ranking fights. Our investigations showed no significant connection between evidence of *M. suis* and the gender of the *M. suis*-infected

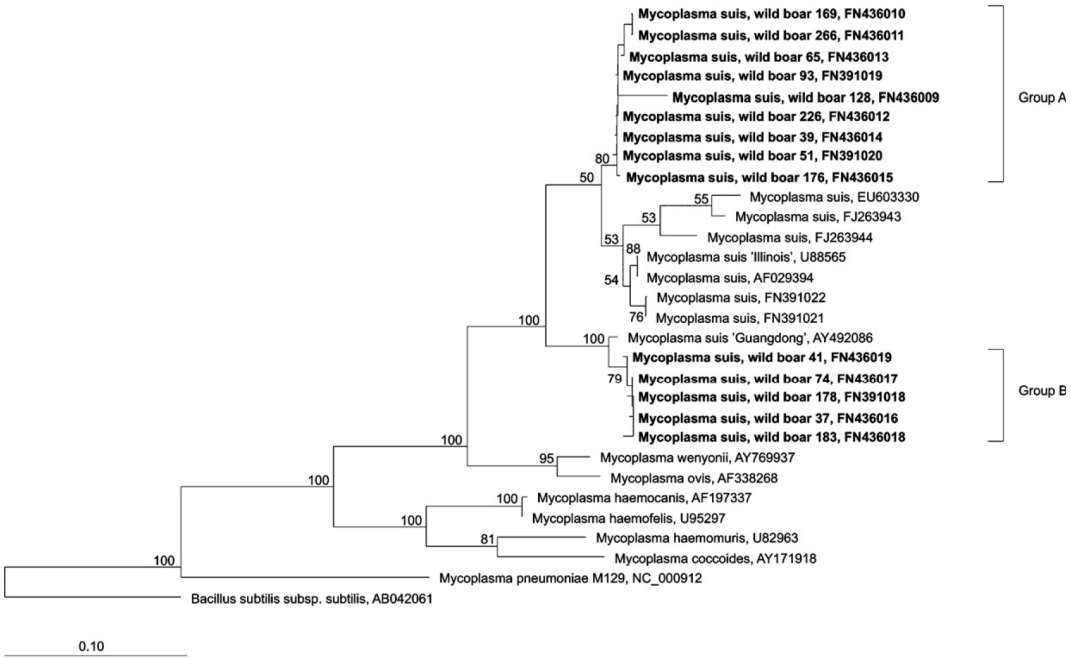

Fig. 1. Phylogenetic analysis of the 16S rRNA gene sequences showing the position of the wild boar isolates among hemotrophic mycoplasmas. The phylogenetic tree was constructed using the neighbor-joining method. Species names and accession numbers in GenBank are given at each axis. The wild boar isolates are in bold. Grouping of the wild boar isolates was indicated with brackets. *Bacillus subtilis* and *M. pneumoniae* served as outgroups. Bootstrap values are given at the nodes of the tree (%). The scale bar indicates estimated evolutionary distance.

wild boars. A similar phenomenon could also be observed with other infectious agents, e.g. porcine circoviruses (Vicente et al., 2004). These distinct epidemiological situations are clearly reflected by our data: the *M. suis* prevalence showed variations of between 0% and 17.82%. As expected, we found a distinctly higher occurrence in the wild boar enclosure (17.82%) in comparison to the hunting grounds (2.70% of all animals from hunting grounds were *M. suis* positive). *M. suis* could not be detected in six of the ten hunting grounds.

The role of wild boars as hosts for other porcine infectious agents is well established. Wild boars, for instance, are hosts of European swine fever, Aujeszky's disease or brucellosis (Heinritzi et al., 1999; Al Dahouk et al., 2005; Ruiz-Fons et al., 2008). Direct contact between domestic pigs in closed stables and wild boars is unlikely. Therefore, spreading of diseases via vectors is more probable. Under experimental conditions *M. suis* infections can be transmitted from pig to pig by hog lice (*Haematopinus suis*), mosquitoes (*Aedes aegypti*), and flies (Prullage et al., 1993). Furthermore, humans who have contact with both, domestic pigs and wild boars, are possible transmitters of infectious agents.

The quantification of *M. suis* in the blood samples showed maximum values of approx. 10^6 *M. suis* per ml of blood. It is unknown whether such *M. suis* blood loads can lead to clinical symptoms in infected wild boars, e.g. anemia. However, it can be assumed that – similar as with domestic pigs – an *M. suis* infection in wild boars can lead to a higher susceptibility for other infectious diseases (Hoelzle, 2008). A pathologic-anatomical and histological investigation of the animals was not possible. Therefore, no information on possibly associated disease patterns is available.

Interestingly, comparative analysis of the 16S rDNA sequences revealed the existence of two different *M. suis* subtypes within the wild boar population. The sequences of the first subtype (group A isolates) were nearly identical to those *M. suis* 16S rDNA sequences found among domestic pigs in Germany and the U.S. Contrary to this, the second subtype (group B isolates) could so far be determined only among domestic pigs in China (Zhou et al., 2009). The result of the sequence analysis reflects the result of the phylogenetic analysis: group A of the wild boar isolates were very closely related to those isolates identified in domestic pigs in Germany. Group B of the wild boar isolates formed an independent sub-cluster within the *M. suis* group together with the Chinese isolate.

To our knowledge, this is the first report on the occurrence of *M. suis* in wild boars. Although the general prevalence and pathogenic potential of *M. suis* in wild boars cannot be assessed on the basis of the present data, wild boars can be considered carriers of *M. suis* as well as a reservoir for cases of transmission to domestic pigs. Nevertheless, additional studies will be necessary for a detailed evaluation of the interaction of *M. suis* between domestic pigs and wild boars. In particular, clarification on whether different subtypes can also be found among domestic pigs in Europe is necessary. Subsequent experimental infections of domestic pigs could provide insight into possible virulence differences of these subtypes.

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VIII.1.2 DIECKMANN *et al.* (2010): Haemotrophic *Mycoplasma* infection in horses

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Short communication

Haemotrophic *Mycoplasma* infection in horses

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ABSTRACT

Haemotrophic mycoplasmas (HM) are parasites on the surface of red blood cells and known to infect a wide range of animals. However, there are no previous evidences of HM infections in horses. In this study HM were detected for the first time in the blood of two horses suffering from poor performance, apathy, weight loss, and anaemia. Using a HM specific PCR assay and subsequent sequencing the infective agents isolated from the blood of said horses were confirmed as closely related to the HM species *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemobos'.

1. Introduction

Haemotrophic mycoplasmas (HM; formerly classified as *Eperythrozoon* and *Haemobartonella*) are uncultivable bacteria, which parasitize erythrocytes of a variety of mammalians, such as pigs, cattle, cats and dogs (Hoelzle, 2008; Groebel et al., 2009). Significance of HM as pathogens of several animal species was previously reported. Animal infections with HM are clinically marked either by an overt live-threatening haemolytic anaemia or a subtle chronic anaemia, by illthrift, infertility, and immune suppression (Messick, 2004; Hoelzle, 2008).

To date almost nothing is known about HM infections in horses. One indication of a possible incidence of equine HM infections is provided by a 30-year-old report on a case of equine "haemobartonellosis" in Nigeria. Affected horses exhibited clinical signs including fever, apathy, lymphadenitis, circulatory disorders, and pale mucosa. Blood smears from these animals revealed bacteria of approx. 0.3 μm on the surface of erythrocytes (Gretillat, 1978).

In this report we provide the first molecular proof that HM infections do occur in horses.

2. Materials and methods

2.1. Animals and samples

Two horses (Hanoverian mares aged 11 and 18 years) from Northern Germany, Luneburg Heath, were presented for clinical examination. EDTA-anticoagulated blood from these horses was subjected to haematological and bacteriological investigation. Blood smears were stained with Giemsa and acridine orange and examined microscopically.

2.2. Molecular analysis: PCR and sequencing

Genomic DNA was extracted as described elsewhere (Hoelzle et al., 2003). To screen specimens for HM a real time SYBR Green PCR assay being able to detect the entire group of known HM was performed according to Willi et al. (2009). For sequencing purposes PCR amplification of the

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16S rDNA was performed using the HotStarTaq Polymerase (Qiagen, Hombrechtikon, Switzerland) and oligonucleotides targeting 16S rDNA regions specific for HM (16S_HAEMOforw: GGCCCATATTCCT(AG)CGGGAAG; 16S_HAEMOrev AC(AG)GGATTACTAGTGATTCCA; Hoelzle et al., 2010). Sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany). The 16S rDNA sequences obtained were compared with each other and to GenBank entries using the BLAST tool provided by NCBI (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and the FASTA algorithm (Biocomputing service, University of Zurich, http://www.bio.unizh.ch/).

2.3. Phylogenetic analyses

To classify the sequences derived from equine blood phylogenetic analyses were carried out applying the ARB software package (Ludwig et al., 2004). For tree calculation the sequences of the two novel equine isolates as well as sequences of selected *Mycoplasma* species and *Bacillus subtilis* were integrated into an ARB database and aligned using the ClustalW tool. Minimum-similarity filters were calculated retaining only positions conserved in at least 0%, 25% or 50% of the selected sequences. Phylogenetic analyses were performed applying each filter in combination with each of the following treeing methods: distance matrix methods (Phylip NEIGHBOR), maximum parsimony (Phylip DNAPARS), and maximum likelihood (RAxML). In case of maximum parsimony and likelihood analyses trees were re-sampled 1000 times by bootstrapping.

2.4. Nucleotide sequence accession numbers

The 16S rDNA sequences obtained from both horses were deposited at GenBank database with the following accession numbers: FN421445 and FN421443.

3. Results

3.1. Clinical, haematological, bacteriological and microscopic findings

The horses were reported by their owners to show unthriftiness, loss of weight and condition, segregation of flock, and tucked up belly. Clinical examination revealed horses suffering from apathy and rough hair. All other physical examination findings e.g. temperature, pulse and respiratory rate were within normal limits. Haematological analysis revealed a decreased haematocrit of 28% and 30% (normal range 31–45%). Other haematological parameters were within normal ranges. Bacteriological cultures yielded no results. In acridine orange stained blood smears distinct roundish particles of approx. 0.4 μm in diameter were observed on the surface of erythrocytes rendering strong evidence of an infection with HM (Fig. 1).

3.2. PCR results and sequence analysis

Both equine DNA samples reacted positive in the universal HM SYBR Green PCR assay (Willi et al., 2009). Using conventional PCR targeting 16S rDNA, fragments of approx. 900 bp were amplified from the blood of both horses. Both fragments were sequenced. The sequences (isolate designations 30/7 and 32/3; GenBank accession numbers FN421445 and FN421443) showed homologies of 97.8% with each other. Furthermore, the isolates demonstrated the highest homology to *Candidatus* Mycoplasma haemobos (98.1% and 98.3%; Acc. no. EF460765), and to *Mycoplasma haemofelis* (94.0% and 94.4%; Acc. no. U95297).

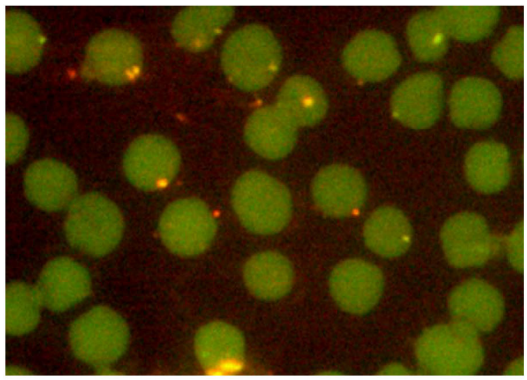

Fig. 1. Blood smear of affected horse stained with acridine orange. Bacterial structures similar to those known from infections with haemotrophic mycoplasmas in other animal species were detected on the surface of erythrocytes (1000× magnification).

3.3. Phylogenetic analyses

Construction of a phylogenetic tree confirmed the relationship to *Candidatus* M. haemobos and *M. haemofelis*. All trees calculated showed stable topologies among each other and were in good agreement with previously published phylogenies of HM (e.g. Neimark et al., 2001; Tagawa et al., 2008). A representative tree is shown in Fig. 2. The novel equine HM isolates clustered within the "haemofelis"-group, which includes amongst others the well established species *M. haemofelis*, *M. haemocanis* (formerly known as *Haemobartonella felis* and *H. canis*) and '*Candidatus* M. haemobos'.

4. Discussion

This study provides the first molecular proof of HM infections in horses using a specific SYBR Green PCR assay and 16S rDNA sequencing. Indications of HM infections in horses based on microscopical findings were reported in 1978 in Nigeria (Gretillat, 1978). Using molecular biological methods the novel equine HM isolates were demonstrated to be closely related to *Candidatus* M. haemobos. Our phylogenetic analyses classified the new equine HM isolates within the so-called "haemofelis"-cluster (Peters et al., 2008), which comprises the representatives of HM formerly classified as *Haemobartonella*. Thus, the novel HM isolates from the horses clearly belong to the cluster comprising those HM formerly classified within the genus *Haemobartonella*. It is noteworthy that already in the first description of equine "haemobartonellosis" in Nigeria in 1978, Gretillat classified the bacteria

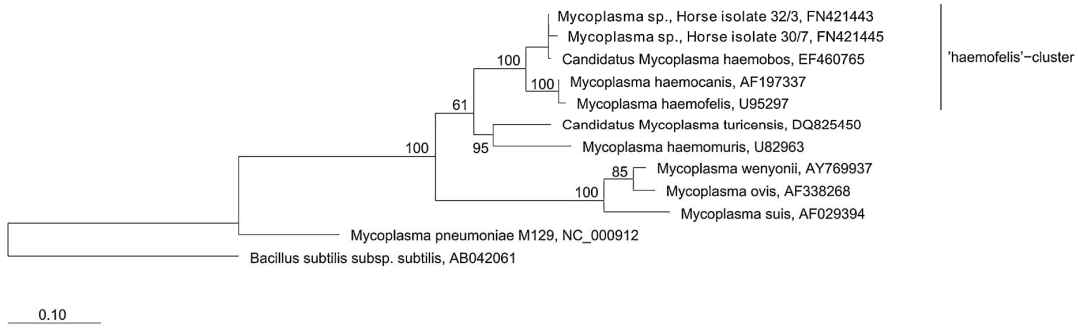

Fig. 2. Comparative sequence analysis using 16S rDNA sequences showing the position of the two equine haemotrophic isolates among the haemotrophic *Mycoplasma* (HM) group. They clearly belong to the so-called "haemofelis"-cluster (Peters et al., 2008), which comprises HM representatives formerly classified as *Haemobartonella*. All trees calculated showed stable topologies. The tree shown was produced applying the maximum likelihood RAxML method (25% SAI, ARB software package; Ludwig et al., 2004). For reconstruction of trees sequences of selected *Mycoplasma* species and *Bacillus subtilis* were included (GenBank Acc. no. are given behind the strain designation). The numbers at the nodes indicate bootstrap values in percent (1000 bootstraps). The bar represents the estimated evolutionary distance.

on the surface of the equine erythrocytes by means of morphological criteria as *Haemobartonella*. It is of particular interest to note that clinical signs observed in the Nigerian horses affected by *Haemobartonella*-like bacteria closely match with the clinical signs reported in the German horses investigated here. This argues for a pathogenic role of HM in horses. The two horses presented here were affected by a syndrome characterised by unspecific signs including anaemia. No evidence for infections with other anaemia-associated agents e.g. *Theileria equi* or *Babesia caballi* was found in Giemsa stained blood smears (data not shown). Other bacteria than haemotrophic mycoplasmas could be excluded as aetiological cause since PCRs targeting universal 16S rDNA regions revealed no other results. Infections with the equine anaemia virus could be excluded, too, due to clinical and laboratory findings. In our case the horses were not specifically treated since an effective anti-mycoplasmal therapy using tetracycline is discussed controversially in horses because of concerns over potentially fatal adverse gastrointestinal effects (Dowling and Russel, 2000). The horses merely received a diet rich in vitamins and minerals and recovered completely 3–6 months later. In future, the etiologic and pathogenic significance of HM infections in horses needs to be elucidated as the symptomatology is rather unspecific.

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VIII.1.3 HOELZLE *et al.* (2011): Detection of *Candidatus* Mycoplasma haemobos in cattle with anaemia

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Short Communication

Detection of *Candidatus* Mycoplasma haemobos in cattle with anaemia

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ABSTRACT

Haemotrophic mycoplasmas are unculturable eperythrocytic pathogens that are found in a wide range of domestic and wild animals. In this study an outbreak of haemotrophic mycoplasmosis in cattle herds in Northern Germany is reported. Affected animals exhibited anaemia and depression and infection was confirmed following microscopic examination of blood smears and on PCR. Sequence analysis indicated that in addition to infection with *Mycoplasma wenyonii*, animals were infected with a novel bovine haemotrophic mycoplasma *Candidatus* Mycoplasma haemobos.

Introduction

Infection of cattle with haemotrophic mycoplasmas (HM) are long-established with bovine infection with *Mycoplasma wenyonii* (formerly *Eperythrozoon wenyonii*), which was first recognised in 1934 (Adler and Ellenbogen, 1934). Nonetheless, our knowledge of the epidemiology and other aspects of these organisms (and their associated diseases) is incomplete.

M. wenyonii belongs to a group of uncultivated HM that attach to and grow on the surface of red blood cells (Neimark et al., 2001). Typically, infections with this organism are latent producing a low-grade parasitaemia and mild anaemia. Occasionally, infected cattle die or may exhibit acute clinical signs (Neimark et al., 2001). Swollen limbs, scrotal oedema, fever, poor coat condition, decreased milk production, weight loss, infertility and unthriftiness have all been reported in infected dairy cattle (Smith et al., 1990; Montes et al., 1994). Traditional diagnostic methods such as the microscopic examination of acridine orange- or Giemsa-stained blood smears are of low sensitivity and specificity (Ritzmann et al., 2009). In consequence PCR-based methods are now the diagnostic method of choice for infections with HM (Ritzmann et al., 2009; Hoelzle et al., 2007).

In this study we investigated an outbreak of infection with HM in cattle on three farms in Northern Germany. The animals presented with anaemia, decreased milk production, infertility and lameness and all herds were free of both bovine viral diarrhoea virus and bovine herpes virus-1. Haematological examination was carried out on 20 animals and blood smears were prepared. Bacterial DNA (Genomic Bacterial DNA Kit, Sigma), was extracted from each of these samples and PCR amplification of HM-specific 16S rDNA performed using HotStarTaq Polymerase (Qiagen) and target region nucleotides.[1] The obtained 16S rDNA sequences were compared to each other and to GenBank entries using the BLASTn/BLASTx and FASTA algorithms (Biocomputing Service, University of Zurich[2]).

Small, coccoid, epicellular bacteria consistent with HM were detected in erythrocytes by fluorescent microscopy on acridine orange-stained blood smears (Fig. 1). Haematocrit and haemoglobin values were below the normal range and 10 animals were positive on PCR. Appropriate sequences were found in the PCR amplicons from the blood samples of five animals (MWG Biotech). Two sequences (designated BovHM3, BovHM8, Accession numbers FN392885 and FN392886) exhibited >99% homology with *M. wenyonii* (Accession number AY769937). The three other isolates (designated BovHM2, BovHM5, BovHM8, Accession numbers FN392887, FN392888 and FN392889) demonstrated the highest homology to the established HM species *M. haemofelis* and *M. haemocanis* (94.4–94.8% identity, Accession numbers U95297 and AF197337) but

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[1] 16S_HAEMOforw: GGCCCATATTCCT(AG)CGGGAAG;16S_HAEMOrev: AC(AG)GGATTACTAGTGATTCCA.
[2] See: www.bio.unizh.ch.

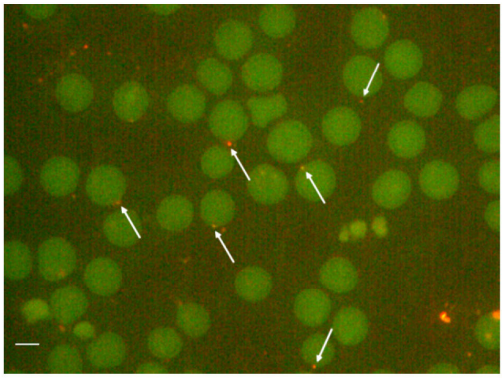

Fig. 1. On microscopic examination of acridine orange-stained blood smears, orange stained bacteria (arrows) were found on the surface of erythrocytes and free in the plasma; bar = 0.5 μM.

only 84.4–85.5% identity to published *M. wenyonii* sequences (Accession number AY769937). Both types of HM were found in two animals.

Phylogenetic analyses were carried out using software package ARB in order to classify this novel mycoplasma sequence (Ludwig et al., 2004). The sequences of five novel bovine isolates as well as of other *Mycoplasma* spp. and of *Bacillus subtilis* were used in the tree calculation. Minimum-similarity filters were calculated retaining only positions conserved in at least 0%, 25% or 50% of the selected sequences. Phylogenetic analyses were performed using each filter in combination with each of the following treeing methods: distance matrix methods (Phylip NEIGHBOR); maximum parsimony (Phylip DNAPARS); and maximum likelihood (RAxML). In the cases of maximum parsimony and likelihood analyses, trees were re-sampled 1000 times by boot-strapping. The calculated trees exhibited stable topology and a representative example is illustrated in Fig. 2. On topological analysis, two phylogenetic groups could be distinguished: those with high homology to *M. wenyonii* belonging to the phylogenetic group containing *M. wenyonii*, *M. ovis*, and *M. suis* and the novel isolates belonging to the 'haemofelis' cluster that includes *M. haemofelis*, *M. haemocanis* and, the as yet unproven, *Candidatus* M. haemobos.

In this study we have identified two genetically distinct HM associated with disease in cattle in Northern Germany. Two of the 10 sequenced isolates were classified as the long established species *M. wenyonii*, while the remaining eight were a novel type of HM. Interestingly, in the pre-PCR/sequencing era, three different HM species were described in cattle: *Eperythrozoon wenyonii* (Adler and Ellenbogen, 1934); *E. teganodes* (Hoyte, 1962); and *E. tuomii* (Uilenberg, 2009). These species differ morphologically and on immunological assays (Uilenberg, 2009). In particular, *E. tuomii* was predominately found in thrombocytes as well as free in plasma. Furthermore, differences have been described in the capacity of these strains to destroy erythrocytes (Uilenberg, 2009). In our study, the novel HM identified appears relatively virulent as indicated by the signs of disease in the investigated herds.

Only *E. wenyonii* (*M. wenyonii*) was included in the 1980 *Approved List of Bacterial Names*. To date, the other species names have not been accepted. The novel HM identified in this study has also been reported in China and Japan (Tagawa et al., 2008) and designated *Candidatus* Mycoplasma haemobos (*C.* M. haemobos) although this taxonomic classification has not been approved. Furthermore, a new bovine HM, closely related to *M. haemofelis*, has been reported in Switzerland (Hofmann-Lehmann et al., 2004). It is now important to clarify whether the HM found in this study, as well as those previously identified (Hofmann-Lehmann et al., 2004; Tagawa et al., 2008), are members of the HM spp. *E. teganodes*/*E. tuomii*, or are in fact a separate, new species. Although the affiliation *C.* M. haemobos to the thrombocyte-associated species *E. tuomii* seems unlikely, both 'old' species should be considered when the definitive classification is made. Currently however, isolates of *E. teganodes* or *E. tuomii* are not available to analyse their 16S rDNA sequences.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

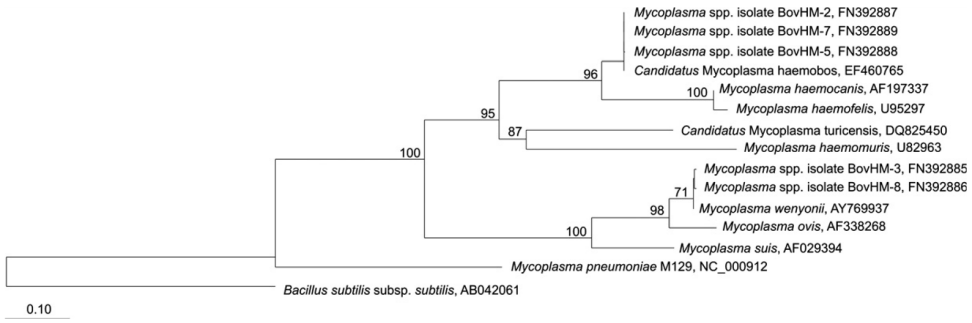

Fig. 2. Phylogenetic analysis of 16S rRNA gene sequences illustrating the position of the bovine haemotrophic isolates among the haemotrophic mycoplasma group. The tree was constructed by using the maximum likelihood method and a minimum similarity filter of 25%. Bootstrap values are given in percent at the tree nodes. Species names and the corresponding accession numbers are given at each axis. Bars represent the estimated evolutionary distance. *Mycoplasma pneumoniae* and *Bacillus subtilis* served as an 'out-group'.

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VIII.1.4 DIECKMANN *et al.* (2011): Significance of haemotrophic mycoplasmas in horses: A disease of young animals?

Significance of hemotrophic mycoplasmas in horses: A disease of young animals?

Hemotrophic *Mycoplasma* infection in horses

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Keywords

Hemotrophic mycoplasma, horse, anemia, real-time PCR, SYBR green, prevalence, microscopy,

Abstract

Hemotrophic mycoplasmas (HM) are small, cell wall-less bacteria and infections are known for a wide range of animals. First indications of HM in Nigerian horses due to microscopy were given in 1978. However the first molecular proof of HM in horses was not reported until 2010, when a fragment of about 900 bp of the 16S rRNA of the equine HM was obtained. This sequence was used for development of a SYBR green I real-time PCR assay specific for equine HM. The lower detection limit of the PCR was 10 genome equivalents per ml of blood. The newly designed assay was successfully applied for the detection and quantification of HM in horses in Germany. A high sample prevalence of 26.5 % (95 %CI: 18.8-35.5 %) was found (31 out of 117 horses). The mean bacterial load was 1.10×10^6 cells/ ml blood (range: minimum 1.05×10^3, maximum 1.27×10^7). Equine HM were also detected by microscopy (Giemsa and acridine orange stained blood smears), but results do not correlate very well with PCR results, as microscopy proved rather unspecific and not sensitive. In horses younger than one year a significant correlation between PCR positive status and anemia was found. No correlation was found in PCR positive animals older than one year. Therefore we assume that HM infection has a higher clinical relevance in young animals.

Introduction

Hemotrophic mycoplasmas (HM) are cell wall-less specialized bacteria which are uniquely adapted to cause eperythrocytic or intraerythrocytic infections in their hosts resulting in deformity and damage of red blood cells (RBCs) (11, 14, 24). Formerly classified as *Haemobartonella* and *Eperythrozoon* species within the order *Rickettsiales*, HM are now classified within the genus *Mycoplasma* based on phylogenetic analysis of the 16S rRNA and RNase P genes (6, 22, 29-33). Infections with HM in pigs, cattle and cats are well-characterized and clinically marked by an overt life-threatening hemolytic anemia or a subtle chronic anemia, ill-thrift, infertility, lethargy, depression, weight loss, growth retardation in young animals and immune suppression. Thereby HM are host-specific and one HM species can only infect a narrow range of animal hosts or even only one animal host species. To date no *in vitro* cultivation system has been established and diagnosis relies mainly upon microscopic evaluation of peripheral blood

smears and PCR techniques (14, 24). In the last years quantitative real-time PCR assays for the specific detection of several HM species have been established. These assays demonstrate an increased sensitivity of diagnostics compared to microscopic detection and conventional PCR techniques (16, 44, 46, 56).

Recently we found the first molecular proof of a novel HM isolate infecting horses. 16S rRNA of this novel equine HM isolate shows identity of 97-98 % to the bovine species '*Candidatus* M. haemobovis' (8). The current knowledge about HM infections in horses is rather restricted, in contrast to the long known HM infections in pigs (14), cattle (24) and cats (54). The only aforementioned report about equine HM was published in 1978, in which 'haemobartonellosis' of horses in Nigeria was microscopically diagnosed (10).

Further studies are necessary to investigate the prevalence and significance of HM infections in horses using specific and sensitive detection methods. Based on the experience with HM infecting other animal species the application of a quantitative real-time PCR assay would be most suitable. Using specific oligonucleotide primers in combination with a SYBR green I real-time PCR assay including a melting curve analysis would provide such a sensitive and specific diagnostic tool and would allow the quantification of the infective agent in blood of affected horses. Due to its possibility for standardization and automation, its reproducibility and its minimal contamination risk (no amplification carry-over), it would provide an inexpensive and reliable diagnostic technique, which could be used in routine diagnostics.

Aims of this study were the determination of occurrence of HM infections in horses, and evaluation of its clinical importance with regard to the induction of anemia. Therefore an equine HM specific quantitative SYBR green I real-time PCR assay for detection of equine HM in horse blood samples was developed.

Material & Methods

Samples

For establishment of the SYBR green I real-time PCR assay, DNA from two HM positive horses (8) and 66 HM negative horses collected from horses presented at the Clinic for Horses (Vetsuisse Faculty, University of Zurich) for unrelated purposes were included. These 66 animals had been analyzed for the presence of HM by a HM-specific universal SYBR green I real-time PCR assay (56).

For screening for equine HM infections, EDTA-anti-coagulated blood samples of 117 horses originating from one breeding farm in Northern Germany with known HM history (8) were collected. This farm takes care of about 120 horses of different ages and breeds. Young horses (9 months to 2 years) were grouped according to their gender and age. During summer young horses are grazed in the marshlands of the river Weser. From October to April young horses are kept at the farm in barns with daily turn-out on pasture. Riding hoses and brood mares stay at the farm throughout the year. Blood samples were collected from 117 horses, thereof were: 70 mares (59.8 %; 19 of them were pregnant (27.1 %)), 17 geldings (14.5 %) and 30 stallions (25.6 %). Horses were of the age of 9 months to 26 years, mean age of horses was 4.3 years. 112 of the horses were warm blood breeds (95.7 %); also there were two German riding ponies (1.7 %), one tinker (0.9 %), one thoroughbred (0.9 %) and one pinto (0.9 %) included into the study. Seven horses (6.0 %) were reported with the following clinical preliminary report: poor nutritional condition, shaggy fur, poor performance.

Laboratory examination of horse blood samples

Hematological parameters, i.e. hematocrit (Ht), hemoglobin (Hb), red blood cell count (RBCC) and white blood cell count (WBCC), were determined (synlab.vet, Geesthacht, Germany) within 24 hours after sampling. Hematocrit was used as parameter of anemia (Ht < 0.32 l/l indicates anemia, reference range: 0.32-0.48 l/l). Giemsa and acridine orange stained peripheral blood smears were prepared.

DNA preparation

Genomic DNA was extracted from 800 µl EDTA-anti-coagulated blood by a direct lysis method (15). Pellets were then used to extract genomic DNA with the GenElute^{TM} Bacterial Genomic DNA Kit (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Genomic DNA was eluted in 200 µl elution buffer and stored at -20 °C until use. To monitor cross-contamination extraction controls consisting of PBS were performed accordingly within each batch of 12 samples.

Standard DNA

Plasmid DNA containing the partial 16S rRNA sequence of the equine HM isolate 30/7, (accession number FN421445, (8)) was used as standard DNA for determination of sensitivity of the assay and for quantification of HM. Cloning was performed using the TOPO TA pCR2.1 cloning kit (Invitrogen, Basel, Switzerland), and the 30/7 plasmid DNA

was purified using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich) according to the manufacturer's instructions. Purity and concentration of the plasmid were checked by agarose gel electrophoresis and optical density measurements (Biophotometer, Vaudaux-Eppendorf). Plasmid size of pCR30/7 of 4.8 kb was used to calculate the concentrations in plasmid copies per micro liter corresponding to genome equivalents (GE) of the equine HM isolate. Dilutions of 10^6, 10^5, 10^4 GE per reaction of the purified plasmid DNA were included as standard controls for HM quantification in each SYBR green I PCR run. Quantitative interpretation of the PCR results was obtained by the LightCycler™ software (Roche Diagnostics, Rotkreuz, Switzerland). The number of bacterial cells in horse blood samples was calculated as follows: cells per ml blood = average GE (determined by LC software) x 50 (16).

SYBR green I real-time PCR assay

Primers were designed for amplification of a 107 bp product of a 16S rRNA region specific for the equine HM isolate (8) using the ARB probe design tool (21). Therefore 16S rRNA sequences of hemotrophic mycoplasmas, non-hemotrophic mycoplasmas and non-mycoplasmal organisms were integrated into an ARB database, aligned using the ClustalW tool and the alignment was refined manually. Accession numbers, primer sequences and part of the alignment are shown in Table 1. Primer binding specificities were tested using pDRAW32 (www.acaclone.com; (47)) and primer sequences were searched for sequence homologies using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi; (2)). Primers were purchased from Eurofins MWG Operon (Ebersberg, Germany).

The LightCycler™ 2.0 System (Roche) was used in combination with the LightCycler® FastStart DNA Master^{PLUS} SYBR green I master mix kit (Roche). Each glass capillary contained 15 µl of master mix (10 µl water, 1 µl primer mix (0.25 µM each), 4 µl premixed master mix (5x)) and 5 µl of extracted template DNA. Cycling conditions were as follows: preincubation (15 min at 95 °C) and 45 cycles of amplification (10 sec 95 °C, 5 sec 55 °C, 20 sec 72°C). A melting curve was generated using the following settings: heating from 65 °C to 95 °C with a ramp rate of 0.1 °C per sec. A no template control (water) was included in each run to check for contamination.

Determination of specificity and sensitivity of the new designed SYBR green I real-time PCR assay

Specificity of assay was tested using DNA from equine HM isolates (8) and HM negative horse samples (n = 66). Other bacterial DNA samples used for specificity testing are listed in Table 2.

The analytical lower limit of detection (LOD) of the SYBR green I PCR assay was determined. Therefore pCR30/7 plasmid DNA was spectrophotometrically quantified and DNA concentrations were adjusted to 53 pg representing 10^7 GE. Ten-fold serial dilutions were analyzed in triplicates. The pCR30/7 plasmid DNA was calculated as 5.3 ag per copy (genome weight = genomic length (bp) x 665 Da/bp x 1.67 x 10^{-24} g/Da (16), size of pCR30/7 plasmid = 4.8 kb).

Determination of intra- and inter-assay variations

Evaluation of the reproducibility of the crossing point (CP) values and melting temperatures (T_M) were performed by analysis of the coefficient of variation (CV). For testing the intra-assay variation three standard series of pCR30/7 plasmid DNA (10^7 to 10^1 GE) were tested within one run. Inter-assay variations were determined by performing three standard curves of pCR30/7 plasmid DNA (10^7 to 10^1 GE) on different days.

Gel electrophoresis, DNA sequencing and sequence analysis

To verify the size of amplicons the SYBR green I real-time PCR were completely applied on a 2 % agarose gel. PCR products were excised from the gel, purified using the QIAquick Gel Extraction Kit (Qiagen AG, Hombrechtikon, Switzerland), cloned (TOPO TA Cloning Kit, Invitrogen) according to the manufacturer's instructions and sequenced (Eurofins MWG Operon). Sequences were searched for homologies using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi; (2)).

Statistical analysis

Data were compiled and analyzed using Excel (Microsoft, Wallisellen, Switzerland) and R Foundation for Statistical Computing (R version 2.11.1; Vienna, Austria). For observed prevalence 95 % confidence intervals (CI) were calculated. Categorical values (gender, anemia, clinical status, pregnancy, type of housing) were analyzed by Fisher's exact test and continuous variables (age, hematological parameter) by the Mann-Whitney *U*-test. Correlation of hematocrit and blood loads was assessed by the spearman rank cor-

relation coefficient (r_s). Differences were considered statistically as significant, if $P \leq 0.05$.

Nucleotide sequence accession numbers

The nucleotide sequences have been deposited at GenBank with accession number FR668084, FR998085 and FR668086.

Results

SYBR green I real-time PCR assay: Primer design and assay setup

Equine HM specific primer sequences are presented in Table 1. The primer design was based on published 16S rRNA gene sequences of HM, selected non-HM, and selected other horse-associated bacteria. At least two mismatches in the forward or reverse primer sequence were found when aligned with the 16S rRNA of nearly all HM species and other bacteria. However, no mismatches were found with the 16S rRNA of the bovine species '*Candidatus* M. haemobovis', as it is nearly identical to the 16S rRNA of the equine HM.

The assay was evaluated as positive, if two criteria were fulfilled: first, an exponential increase in fluorescence acquisition during the first 32 cycles of PCR (CP < 32). Samples with crossing points of 32 to 35 cycles were repeated to verify results. If the CP values were consistently lower than 35 cycles, the sample would be regarded as positive. Second, the melting temperature (T_M) curve showed a distinct peak at $T_M = 81 \pm 1°C$. If the curve was not distinct and/ or a certain T_M determination was not possible, the samples were repeated to verify results. If no amplification occurred, the CP values were > 35 cycles or no distinct T_M curve could be obtained, the PCR reaction was assigned as negative. In all runs the positive controls (pCR30/7 pCR plasmid DNA) were positive and the no template controls (water) were negative. Only PCR runs fulfilling these criteria were taken into consideration.

Evaluation of the SYBR green I real-time PCR assay

The SYBR green I real-time PCR assay reacted positive with DNA from equine HM isolated from horse blood. Also cross-reaction with '*Candidatus* M. haemobovis' was observed as expected. Neither other HM, nor non-HM species, nor non-mycoplasmal organisms gave a positive signal (Tab. 2). Additionally, all universal HM PCR (56) negative horses (n = 66) reacted negative in the novel SYBR green I real-time PCR assay. Presence or absence and size of amplified DNA (107 bp) derived by the SYBR green I

real-time PCR assay was confirmed by agarose gel electrophoresis (Figure 1) and amplicon specificity of the positive control was verified by sequencing showing an overall identity of 100 % with the primer spanned region. Sequencing of three selected PCR products revealed sequences of 105-106 bp showing an identity of 99-100 % to *Mycoplasma* sp. horse isolate 30/7 (Acc. no. FN421445) and 96-98 % identity to '*Candidatus* M. haemobovis' (Acc. no. EF460765).

For analysis of sensitivity ten-fold serial dilutions of pCR30/7 plasmid DNA ranging from 5.3 ag to 53 pg (corresponding to 10^0 to 10^7 GE) per reaction were tested in the SYBR green I real-time PCR assay. PCR reacted positive if at least 10 GE/ ml blood were present.

Intra- and inter-assay variations were measured to determine the reproducibility of the SYBR green I real-time PCR assay. For the intra-assay variation the coefficients of variation (CV) of CP and T_M values were in the range of 0.435 to 9.151 % and 0.019 to 0.070 %, respectively. For the inter-assay variation the CV of CP values varied from 0.143 to 9.873 % and CV of T_M values from 0.031 to 0.418 % (Table 3).

Evaluation of significance of HM infection in horses by SYBR green I real-time PCR

To evaluate significance of HM infection in horses, 117 blood samples of anemic and non-anemic horses were tested. Thirty-one horse blood samples (26.5 %; 95 %CI: 18.8-35.5 %) revealed a positive SYBR green I real-time PCR result showing a mean crossing point of CP = 32.28 ± 1.44 (range: minimum 29.43, maximum 34.69) and a mean melting temperature of T_M = 80.88 ± 0.37 °C (range: minimum 80.16, maximum 81.78). Nine horses showing an anemia (Ht < 0.32; 9/23, 39.1 %), ten horses with a low level hematocrit (Ht = 0.32-0.34; 10/38, 26.3 %) and twelve non-anemic horses (Ht > 0.34; 12/56, 21.4 %) exhibited positive PCR results.

All equine blood samples that tested positive by the newly designed SYBR green I real-time PCR assay were further analyzed for HM quantification. The overall mean bacterial load was 1.10×10^6 cells/ ml blood (range: minimum 1.05×10^3, maximum 1.27×10^7). In anemic and PCR positive horses a mean bacterial load of 1.10×10^6 cells/ ml blood (range: minimum 1.28×10^3, maximum 4.15×10^6) was observed and in non-anemic and PCR positive horses a mean bacterial load of 1.42×10^5 cells/ ml blood (range: minimum 1.05×10^3, maximum 1.27×10^7) was detected. In the group of horses (n = 7) with the preliminary report of showing a reduced performance condition, having a shaggy fur and being meager, two horses showed a positive PCR signal (28.6 %;

95 %CI: 3.7-71.0 %) with a mean bacterial load of 4.65 x 10^3 cells/ ml blood (range: minimum 1.05 x 10^3, maximum 8.25 x 10^3).

Hematological findings

Ht, Hb, RBCC and WBCC were analyzed and compared to reference values (7). Twenty-three of 117 horses (28.2 %) were anemic, whereas 38 more horses (32.5 %) showed a low level Ht within the reference range (0.32-0.34 l/l). Mean Ht was 0.34 l/l (range: minimum 0.26, maximum 0.52). Mainly young horses (< 1 year) were affected from low Ht values (n = 30, mean Ht = 0.31 l/l, range: minimum 0.26, maximum 0.35). They mostly showed a low level Hb concentration (n = 30; mean Hb = 115.3 g/l; range: minimum 94, maximum 129; reference range: 100-180 g/l) and a low level RBCC (n = 30; mean RBCC = 9.1 x 10^{12} cells/l; range: minimum 7.09 x 10^{12}, maximum 10.64 x 10^{12}; reference range: 6.0-12.0 x 10^{12} cells/l), too. Mean Hb of all animals (n = 117) was 128 g/l (range: minimum 94, maximum 212) and mean RBCC was 8.80 x 10^{12} cells/l (range: minimum 6.54 x10^9, maximum 13.38 x 10^9). Some horses exhibited a leucocytosis (n = 16, 13.7 %; mean WBCC = 13.1 x 10^9 cells/l; reference range: 6.0-12.0 x 10^9 cells/l). The overall (n = 117) mean WBCC was 9.6 x 10^9 cells/l (range: minimum 3.6 x 10^9, maximum 14.3 x 10^9).

No evidence for infections with other anemia-associated agents, e.g. *Theileria equi* or *Babesia caballi*, was found in Giemsa stained blood smears. Infections with equine anemia virus could be excluded, too, due to clinical and laboratory findings.

Characteristics of HM PCR positive horses

Looking at the complete population investigated, PCR positive horses had significantly lower RBCC than PCR negative horses ($P = 0.001$; Figure 3, Table 4). In addition, Ht and Hb tended to be lower in PCR positive horses compared with PCR negative animals (Figure 3, Table 4). No significant difference was found in WBCC. However, in horses < 1 year, Ht, Hb, and RBCC were significantly lower in PCR positive compared with PCR negative horses ($P = 0.023$, $P = 0.021$, $P = 0.013$, respectively; Figure 3, Table 4). In contrast, in PCR positive animals > 1 year all measured blood parameters were not significantly different compared with PCR negative horses. PCR positive horses < 1 year showed significantly lower Ht and Hb values than PCR positive horses > 1 year ($P < 0.0001$) and a significantly higher WBCC (P = 0.04), but no significantly changed RBCC (Figure 4). No significant correlations between bacterial blood loads and blood parameters could be found (assessed by the spearman rank correlation coeffi-

cient r_s, Table 4). PCR positive status of the overall population independent from age was not significantly associated with anemia. However, in horses < 1 year anemia was associated with infection. In general, PCR positive and PCR negative horses showed no significant differences in age, gender, pregnancy and clinical status.

Comparison of SYBR green I real-time PCR results to microscopic detection methods

Results of SYBR green I real-time PCR assay were compared to results of microscopy of Giemsa and acridine orange stained peripheral blood smears (Figure 2). Forty-six of 117 samples (39.3 %) revealed HM in peripheral blood smears stained with either Giemsa or acridine orange. In 17 Giemsa (14.5 %) and in 44 acridine orange (37.6 %) stained horse blood smears HM were detected as coccoid structures of approx. 0.3-0.4 µm in size closely attached to the surface of RBCs. In fifteen samples (12.8 %) HM could be detected in both staining techniques and 71 samples (60.7 %) were negative in both staining techniques.

Twenty-five of 46 samples microscopically diagnosed as HM positive (either Giemsa or acridine orange stained) were confirmed as positive by PCR (54.3 %). In 71 microscopically HM-negative samples, six reacted positive in PCR (8.5 %). This corresponds to a rate of false-positive microscopic results of 23.3 % and false-negative microscopic results of 19.4 %.

Discussion

Although first evidence of equine HM infections have been described a long time ago by microscopy (10) our knowledge of HM in horses is rather limited. Recently the molecular confirmation of HM in horses was published for the first time (8).

In this study we describe the development of the first SYBR green I real-time PCR assay for the sensitive and specific detection of HM in the blood of horses and the application of the assay for the detection of HM in a horse population in Germany and analysis of its clinical importance. Formerly the diagnosis of HM infections was based on the microscopic detection in chemically stained blood smears – a method which is not specific and sensitive enough to detect animals with low bacterial blood loads (4, 37, 48). For other HM species, e.g. *M. haemofelis*, *M. suis*, *M. haemocanis* and '*Candidatus* M. turicensis', the establishment of specific and sensitive real-time PCR methods has delivered valuable insights into the significance of HM infections in cats, dogs and pigs (3, 16, 44, 53). Accordingly the reliable quantitative detection of HM infections in horses is the crucial step for the evaluation of the actual significance of equine HM. Our

newly developed SYBR green I real-time PCR assay demonstrated a sensitivity of 10 GE/ ml blood. The analytical specificity of the SYBR green I real-time PCR was confirmed by testing DNA from different bacteria (mycoplasmas and other bacteria with clinical relatedness). As predicted *in silico* only cross reactivity was found with the '*Candidatus* M. haemobovis' DNA.

Comparison of the PCR and microscopic results revealed a poor correlation. To some extent microscopically positive tested animals reacted negative in PCR and vice versa. False-negative microscopic detection may result from low bacterial loads or inapparent infections with bacteria being absent from blood, whereas false-positive microscopic results are because of confusion of HM with Howell-Jolly or Heinz bodies, background debris and staining artifacts (19, 22, 42). It was reported earlier that blood loads of 4.4×10^6 '*Candidatus* M. turicensis' copies/ ml blood correspond to only one bacterium per 10^3 to 10^4 RBCs (26). Bacterial blood loads of affected horses in this study were in the same range hindering the unambiguous identification of HM on the surface of RBCs by microscopy. Using the newly designed SYBR green I real-time PCR assay an unambiguous and sensitive detection of HM in blood of affected horses was possible. The possibility of false-positive or false-negative SYBR green I real-time PCR results is negligible due to its high specificity and sensitivity. However, it remains difficult to establish a novel diagnostics tool, when a good reference method is missing.

The HM prevalence in the horse population investigated in this study was 26.5 % (95 % CI: 18.8-35.5 %). Analogically high HM prevalences of 22.3 % and 18.6 %, respectively, were found in cattle without apparent clinical signs in Japan ('*Candidatus* M. haemobovis', (43)) and in alpacas in Switzerland (18). In comparison, prevalences of feline (0.5 to 8.5 %) (52) and canine HM in Switzerland (1.2 %) (51) are considerably lower. Since our study includes horses of only one farm the general prevalence and pathogenic potential of equine HM cannot be assessed on the basis of the present data. Further studies are necessary to determine the actual prevalence and to analyze, whether the found HM infections may be a regional phenomenon or a single-herd, sporadic event.

Younger horses seem to be more affected by an equine HM infection. Though red blood cell count (RBCC) was significantly lowered in all PCR positive animals, hematocrit (Ht) and hemoglobin (Hb) were also significantly lowered in infected animals < 1 year, but not in infected horses > 1 year (Figure 3 and Figure 4). Therefore, this found correlation in horses < 1 year should be investigated in future, if an equine HM infection has a more severe course of disease in young horses, as it was described for HM infection in

llamas (23, 35) and sea lions (49). Overall looking at the entire population independent of age, equine HM infection was not associated with anemia and blood loads do not correlate with Ht values. Absence of correlation of anemia and HM infection was also reported for feline HM (52), whereas *M. suis* infection in pigs is often accompanied by severe anemia and strong correlation of Ht, Hb and RBCC (14,37).

In horses, HM infection most often seems to have a subclinical or asymptomatic course of disease showing only low bacterial loads in blood. For this reason detection of HM in horse blood as well as assignment of a distinct clinical manifestation is complicated. Mostly, horses only show unspecific clinical signs like slimming, shaggy fur, apathy and a bad general performance.

HM were detected by SYBR green I real-time PCR in healthy horses without any clinical signs and without showing abnormalities in the blood count. A similar situation was reported for '*Candidatus* M. haemolamae' and *M. haemocanis*. Disease and anemia seem to be developed only by stressed, immunocompromised or co-infected animals (19, 24, 48). For *M. wenyonii* it was described that clinical signs were more present in combination with concurrent infections with *Anaplasma marginale* (17). An undulating course of infection alternating between pronounced clinical signs and an asymptomatic stage was described for feline HM and *M. suis* (14, 54). Therefore it can be assumed that bacterial loads near the detection limit do not play an important clinical role. Nevertheless the balance of bacteria and host immune system may be disturbed by stress or concurrent infections and an asymptomatic infection may turn into a clinical manifestation of disease with more or less pronounced clinical signs.

Interestingly, infections of cattle herds with a rather similar HM species ('*Candidatus* M. haemobovis') were reported in the same region (13). Both, equine and bovine HM isolate, exhibits high 16S rRNA sequence identity (97-98 %). According to the cut-off values (9) this sequence difference may be not enough for designation of a novel species. Therefore it could be speculated that the equine HM isolate and '*Candidatus* M. haemobovis' could be one species cross-infecting cattle and horses. Host specificity of mycoplasmas was recently questioned (34). In respect of historical reasons HM species were named after the animal species they were isolated from. A similar situation as in horse and cattle is given with *M. haemofelis* (cat) and *M. haemocanis* (dog), which shows a 16S rRNA sequence identity of 99.3-99.7 %, but only 94.3-95.5 % sequence identity of the RNase P (*rnpB*) gene (33, 41). Further phylogenetic analyses (e.g. sequencing of the *rnpB* gene) and cross-infectivity studies are necessary to evaluate, if the

novel equine HM isolate is an own species with the proposed designation '*Candidatus* Mycoplasma equi'. Unfortunately amplification and sequencing of the full length 16S rRNA and the *rnpB* gene failed during this study as well as in our previous study (8).

In conclusion we confirmed HM infections in Northern Germany using our newly designed SYBR green I real-time PCR assay. The clinical significance of equine HM infections still remains difficult to interpret and only in horses younger than one year equine HM infection was associated with anemia. More investigations in the field of clinical characterization of this novel infection in horses have to be done in future to establish a clear clinical picture and to examine the pathogenic potential of HM infection in horses. The herein developed SYBR green I real-time PCR assay provides an important prerequisite for doing so.

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Tables

Table 1 Alignment of primer sequences with target and non target species 16S rRNA sequences of closely related organisms.

Species	Forward primer 5‘-3‘	Reverse primer 5‘-3‘	Acc. No.	Reference
Primer sequence	CAGGCGGATGTGTAAGTTC	CGCCTCCGGTGTTCTTAAAC		
Novel equine HM isolate 30/7	-------------------	--------------------	FN421445	(8)
‘*Candidatus* Mycoplasma haemobovis’	-------------------	--------------------	EU367965	(42)
Mycoplasma haemofelis	-------------------	------T----------G-	AY150984	(45)
‘*Candidatus* Mycoplasma haemominutum’	T-------CAAA–TGA-CT	A-----T-------C–C--T	DQ157149	(52)
‘*Candidatus* Mycoplasma turicensis’	----------AA-------	------T-----------T	DQ825450	(55)
Mycoplasma haemomuris	---------TG--------	------T---------G--T	U82963	(36)
Mycoplasma coccoides	----------AAC------	------T-----------T	AY171918	(32)
Mycoplasma suis	T----T--A----GTA-C-	A-----T-------C–C--T	U88565	(36)
Mycoplasma wenyonii	T------GGAG--TGA-C-	A-----T-------C–C--T	AF016546	(31)
Mycoplasma ovis	T------GGAG-CTGA-C-	A-----T-------C–C--T	AF338268	(28)
‘*Candidatus* Mycoplasma haematoparvum’	T-------CAA--TGA-GT	A-----T-------C-CCA-	AY532390	(20)
‘*Candidatus* Mycoplasma haemodidelphis’	T-------CAGTCTGA-C-	-----TT-------C–C--T	AF178676	(25)
‘*Candidatus* Mycoplasma kahanei’	T-------GAGT–TGA-CT	A-----T-------C–C--T	AF338269	(27)
‘*Candidatus* Mycoplasma haemolamae’	T---T---G–A--TGA-C-	A-----T-------C–C--T	AF306346	(25)
Mycoplasma pneumoniae	---------TGAA----CT	----A-T-------C–TC-T	NC_000912	(12)
Mycoplasma arginini	T----T–T-TAT-----CT	-----TT---------CC-T	M24579	(50)
Mycoplasma penetrans	---------T-AC----CT	------T---------CC-T	NC_004432	(39)
Escherichia coli	-------T-TGT-----CA	----A-----A---C–CC-G	U18997	d.s.
Bacillus cereus	----T--T-TCT-----CT	----A-T-------C-CC-T	AY138272	(38)
Bacillus subtilis	-------T-TCT-----CT	----A-T-------C-CC-T	AB042061	d.s.
Salmonella enterica	-------T-TGT-----CA	----A-----A---C–CC-G	U92197	d.s.
Bartonella quintana	T--------ATT-----CA	----A-T-------C-CCGA	BX897700	(1)
Streptococcus equi	-------T-TGA-----CT	----A---------C–CC-T	DQ303186	(5)
Clostridium perfringens	T---------AT-----GG	----A-T---------CCTA	AB045286	(40)

Table 2 Bacterial strains used for evaluation of specificity of the newly designed SYBR green I real-time PCR assay

Species	Origin	Result
Hemotrophic mycoplasmas		
Horse isolate No. 30/7	Field strain[a]	Positive
Horse isolates	Field samples, n = 117	31 positive
Horse blood controls	Field samples[b], n = 66	Negative
'*Candidatus* Mycoplasma haemobovis'	Field strains[c], n = 6	Positive
Mycoplasma haemofelis	[d]	Negative
Mycoplasma haemominutum	[d]	Negative
'*Candidatus* Mycoplasma turicensis'	[d]	Negative
Mycoplasma wenyonii	Field strains[c], n = 4	Negative
Mycoplasma suis	Strain 3808[e]	Negative
Mycoplasma suis	Strain 3806[e]	Negative
Mycoplasma suis	Strain 146[e]	Negative
'*Candidatus* Mycoplasma haemolamae'	Field strains[f], n = 5	Negative
Non-hemotrophic mycoplasmas		
Mycoplasma arginini	[g]	Negative
Mycoplasma penetrans	[g]	Negative
Mycoplasma pneumoniae	Sebastian Schmidl[h]	Negative
Non-mycoplasma organisms		
Bacillus cereus	isolated from horse[g]	Negative
Bartonella bacilliformis	Christoph Dehio[i]	Negative
Bartonella henselae	Christoph Dehio[i]	Negative
Bartonella quintana 'Fuller'	Christoph Dehio[i]	Negative
Bartonella quintana 'Toulouse'	Christoph Dehio[i]	Negative
Bartonella tribocorum	Christoph Dehio[i]	Negative

Clostridium perfringens Type A	isolated from horse[g]	Negative
Escherichia coli	isolated from horse[g]	Negative
Salmonella sp.	isolated from horse[g]	Negative
Streptococcus equi	isolated from horse[g]	Negative

[a] Field strain isolated from an anemic horse (8), used as positive control for the SYBR green I real-time PCR assay (see material and methods); [b] DNA of horse blood samples collected from horses presented at the Clinic for Horses, Equine Department, Vetsuisse Faculty, University of Zurich, for unrelated purposes; negative for any HM infection (SYBR green I real-time PCR assay covering any HM species (56)); [c] Field strains isolated from anemic cattle (13); [d] DNA of three feline HM isolates from anemic cats (53, 55, 57); [e] Blood from splenectomized pigs experimentally infected with *M. suis* (Institute of Veterinary Bacteriology, University of Zurich); [f] Field strains isolated from anemic alpacas (Hoelzle et al., manuscript in preparation); [g] Strain collection of Institute of Veterinary Bacteriology; [h] provided by Sebastian Schmidl (Department of General Microbiology, Georg-August-University of Göttingen); [i] DNA provided by Christoph Dehio (Infection Biology, Biozentrum, University of Basel)

Table 3 Inter-assay and intra-assay reproducibility of the newly designed SYBR green I real-time PCR assay

	Inter-assay variation				**Intra-assay variation**			
Copy number[a]	**Mean CP (± SD)[b]**	**CV (%)[c]**	**Mean T_M (± SD)[b]**	**CV (%)**	**Mean CP (± SD)[b]**	**CV (%)**	**Mean T_M (± SD)[b]**	**CV (%)**
10^7 GE[d]	22.46 ± 0.032	0.143	81.19 ± 0.092	0.126	22.34 ± 0.097	0.435	81.32 ± 0.031	0.038
10^6 GE	26.32 ± 0.233	0.885	81.17 ± 0.076	0.093	26.21 ± 0.010	0.038	81.26 ± 0.030	0.037
10^5 GE	28.89 ± 0.586	2.029	81.19± 0.025	0.031	29.10 ± 0.127	0.435	81.18 ± 0.025	0.031
10^4 GE	26.57 ± 0.385	1.449	81.17 ± 0.040	0.050	26.36 ± 0.537	2.036	81.20 ± 0.015	0.019
10^3 GE	32.78 ± 2.874	8.766	81.26 ± 0.096	0.119	34.28 ± 0.212	0.619	81.30 ± 0.057	0.070
10^2 GE	28.24 ± 2.517	8.914	81.20 ± 0.121	0.150	28.02 ± 2.564	9.151	81.38 ± 0.038	0.047
10^1 GE	31.37 ± 3.097[e]	9.873	81.22 ± 0.339[e]	0.418	33.56[f]	n.d.[g]	81.46[f]	n.d.

[a] Copy number were calculated from spectrophotometrically quantified DNA as described in the material and methods section; [b] Mean crossing point (CP), mean melting temperature (T_M) and standard deviations (SD) were calculated from triplicates of each concentration; [c] coefficient of variation; [d] genome equivalents; [e] only 2/3 reactions were positive; [f] only 1/3 reactions were positive; [g] not determined

Table 4 Hematological values of PCR positive and PCR negative horses

Variable	PCR negative (n = 86)		PCR positive (n = 31)			
	Median	**95 % CI[a]**	**Median**	**95 % CI**	**Reference range[b]**	***P***
Ht[c]	0.35	0.32-0.37	0.34	0.31-0.36	0.32-0.48 l/l	0.133
Hb[d]	129.50	120.25-137.75	124.00	115.50-132.50	100-180 g/l	0.069
RBCC[e]	8.89	8.39-9.52	8.42	7.92-8.97	6.0-12.0 x 10^{12} cells/l	0.001*
WBCC[f]	9.80	7.93-11.18	9.20	7.45-11.10	6.0-12.0 x 10^{9} cells/l	0.700
	PCR negative, > 1 year (n = 64)		**PCR positive, > 1 year (n = 23)**			
	Median	**95 % CI**	**Median**	**95 % CI**	**Reference range**	***P***
Ht	0.36	0.34-0.38	0.35	0.34-0.37	0.32-0.48 l/l	0.196
Hb	133.00	126.00-140.00	129.00	124.00-135.50	100-180 g/l	0.105
RBCC	8.81	8.29-9.25	8.42	7.95-8.94	6.0-12.0 x 10^{12} cells/l	0.094
WBCC	9.30	7.58-10.93	8.30	7.35-10.75	6.0-12.0 x 10^{9} cells/l	0.652
	PCR negative, < 1 year (n = 22)		**PCR positive, < 1 year (n = 8)**			
	Median	**95 % CI**	**Median**	**95 % CI**	**Reference range**	***P***
Ht	0.31	0.30-0.33	0.30	0.29-0.31	0.32-0.48 l/l	0.023*
Hb	118.50	111.25-123.00	110.50	106.75-113.75	100-180 g/l	0.021*
RBCC	9.41	8.82-9.86	8.43	7.84-9.06	6.0-12.0 x 10^{12} cells/l	0.013*
WBCC	10.75	9.80-11.65	11.25	8.80-11.68	6.0-12.0 x 10^{9} cells/l	0.593

Results of hematological analysis are shown for all horses and for horses grouped according to their age (> 1 year; < 1 year); [a] confidence interval; [b] Reference range according to (7);[c] hematocrit; [d] hemoglobin; [e] red blood cell count, [f] white blood cell count; * significant if $p \leq 0.05$

Figures

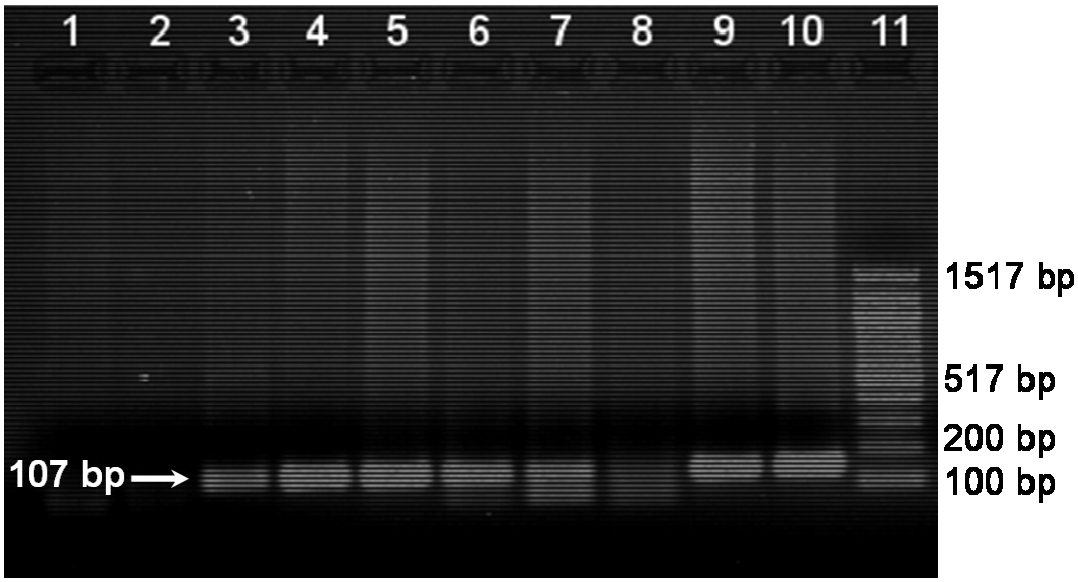


Figure 1 SYBR green I real-time PCR assay of 10 samples applied on an 2% agarose gel. An amplicon of the corresponding size of 107 bp was obtained in PCR positive horses (lane 3 = no. 90; 4 = no. 103; 5 = no. 108; 6 = no. 111; 7 = no. 114; 9 = no. 126; 10 = no. 127) and amplification was absent in no template control (lane no. 1) and PCR negative horses (lane 2 = no.86; lane 8 = no. 117). 100 bp ladder (lane 11; New England Biolabs, Bioconcept, Allschwil, Switzerland) was used for accession of amplicon sizes.

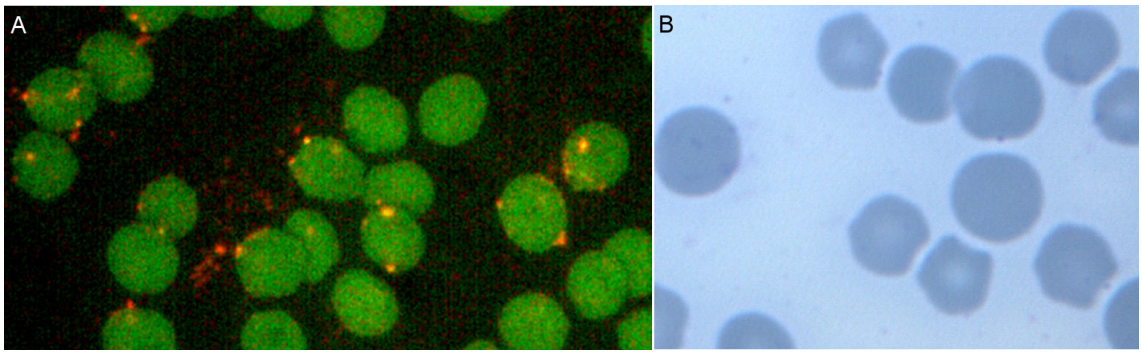
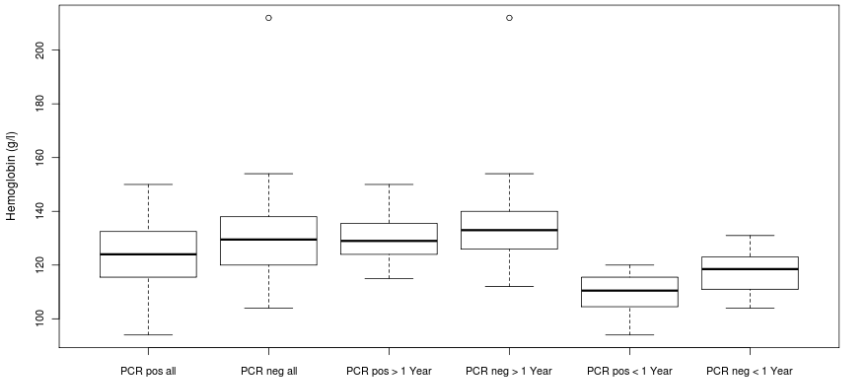
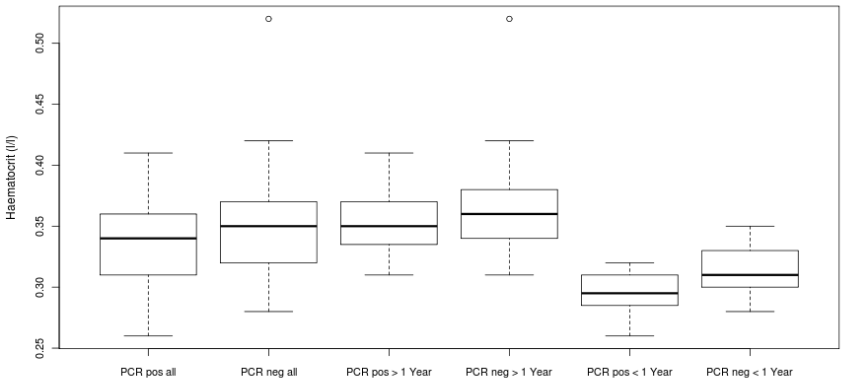
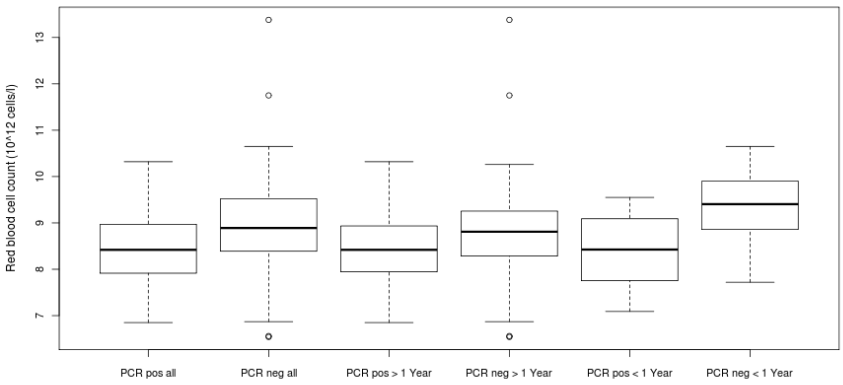


Figure 2 Micrographs of Giemsa (A) and acridine orange (B) stained peripheral blood smears (magnification 1000x) of a horse infected with hemotrophic mycoplasmas. Roundish particles of approx. 0.3-0.4 µm in size are detected on the surface of red blood cells in both staining techniques. In Giemsa stained blood smear it can be seen, that some hemotrophic mycoplasmas are laying in small grooves on the surface of the erythrocytes.







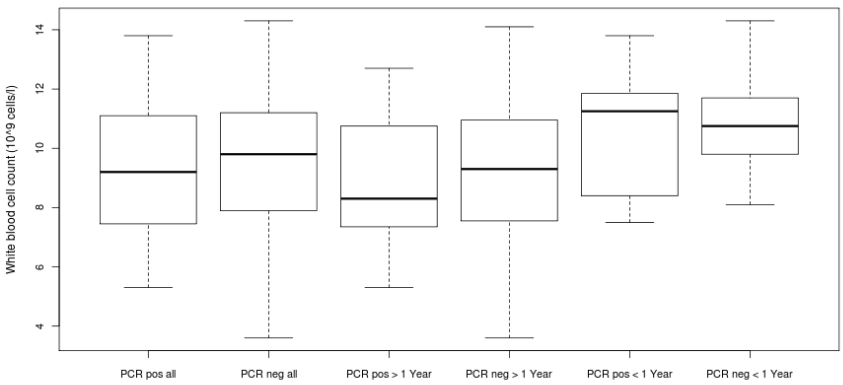


Figure 3 Blood parameters ((A) hemoglobin, (B) hematocrit, (C) red blood cell count, (D) white blood cell count) of PCR positive (PCR pos) and PCR negative (PCR neg) horses are presented as box plots. Results are shown for all horses and for horses grouped according to their age (< 1 year, > 1 year). Boxes extend from the 25th to the 75th percentile, median is presented as horizontal line and the bars extend to the minimum and maximum value. Dots represent outliers. Values from PCR positive and PCR negative horses were analyzed for significant differences using the Mann-Whitney *U*-test. *P*-values are listed in Table 4.

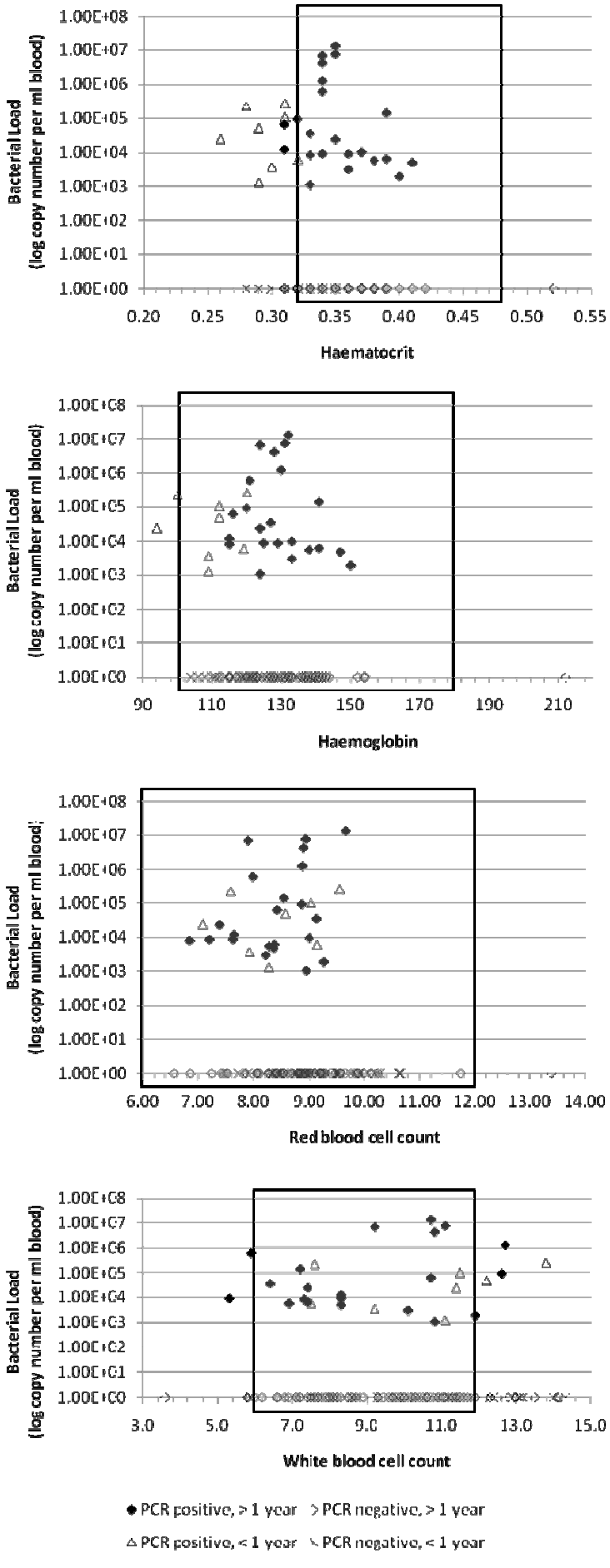


Figure 4 Comparison of correlation between blood parameters ((A) hemoglobin, (B) hematocrit, (C) red blood cell count, (D) white blood cell count) and bacterial load in PCR positive horses > 1 year (n = 23) and PCR positive horses < 1 year (n = 8)). Grey boxes indicate the corresponding reference ranges according to Cowell &Tyler (7). Differences are significant, if $P \leq 0.05$.

VIII.2 Detailed presentation of samples

Table VIII-1 Detailed presentation of blood samples collected during this study

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood anti-coagulant	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
001	Dacana	2003	M	n/a	H	18.02.08	MD	AL	+	-	-	-	HM+, 15.01.08
				yes		16.11.09	MD	EDTA	+	+	-	+	
002	Shirley	1997	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				no		16.11.09	MD	EDTA	+	+	-	+	
003	Bretagne	1992	M	n/a	H	18.02.08	MD	EDTA	-	-	-	-	
				no		16.11.09	MD	EDTA	+	+	-	+	
				no		09.12.09	MD	Citrate	-	+	+	+	
004	Weltfee	1999	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				yes		16.11.09	MD	EDTA	+	+	-	+	
005	Emelie	2002	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				yes		16.11.09	MD	EDTA	+	+	-	+	
006	Leca	2002	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				yes		16.11.09	MD	EDTA	+	+	-	+	
007	Royal Star	2003	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				yes		16.11.09	MD	EDTA	+	+	-	+	
008	Pussy Cat	1987	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
009	Lawenna	1989	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
010	Dipsy	2004	M	n/a	H	18.02.08	MD	AL	+	-	-	-	HM+, 14.11.07
				no		16.11.09	MD	EDTA	+	+	-	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
011	Kaja	2002	M	n/a	T	18.02.08	MD	EDTA	+	-	-	-	HM+, 15.01.08
				no		16.11.09	MD	EDTA	+	+	-	+	
012	Darcy	2003	G	/	H	18.02.08	MD	EDTA	+	-	-	-	
						16.11.09	MD	EDTA	+	+	-	+	NC 4, lazy, shaggy
013	Dante	2005	G	/	H	18.02.08	MD	EDTA	+	-	-	-	
						15.05.08	MD	EDTA	+	-	+	-	
014	Kassiopeia	1992	M	n/a	T	18.02.08	MD	EDTA	+	-	-	-	
				n/a		15.05.08	MD	EDTA	+	-	+		
				no		16.11.09	MD	EDTA	+	+	-	+	
015	Wolkenfee	1998	M	n/a	H	18.02.08	MD	EDTA	+	-	-	-	
				no		17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
016	Pagina	1987	M	no	T	18.02.08	MD	EDTA	+	-	-	-	HM+, 09.02.08
				no		15.05.08	MD	EDTA	+	-	+	-	
017	Lucky	1998	G	/	H	19.02.08	MD	EDTA	+	-	-	-	HM+, 18.02.08
						15.05.08	MD	EDTA	+	-	+	-	
						25.07.08	MD	EDTA	+	-	+	-	
018	Don Giovanni	2005	S	/	O	20.02.08	MD	EDTA	+	-	-	-	
019	Don Fidelio	2005	S	/	H	20.02.08	MD	EDTA	+	-	-	-	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
020	Escot	2006	S	/	H	20.02.08	MD	EDTA	+	-	-	-	
			G	/		18.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
021	Lilli	2004	M	n/a	H	20.02.08	MD	EDTA	-	-	-	-	
				yes		16.11.09	MD	EDTA	+	+	-	+	
022	Dondra	2005	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				n/a		15.05.08	MD	EDTA	+	-	+		
				yes		16.11.09	MD	EDTA	+	+	-	+	
023	Dorit	2006	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				yes		17.11.09	MD	EDTA	+	+	-	+	
024	Tristan	1998	G	/	F	20.02.08	MD	EDTA	+	-	-	-	
						15.05.08	MD	EDTA	+	-	+		
						18.11.09	MD	EDTA	+	+	-	+	
025	Caray	1995	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				no		16.11.09	MD	EDTA	+	+	-	+	
026	Rubina	2000	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				no		18.11.09	MD	EDTA	+	+	-	+	
027	Watzlaff	1988	G	/	H	20.02.08	MD	EDTA	+	-	-	-	
028	Kensington	2004	G	/	H	20.02.08	MD	EDTA	+	-	-	-	
						15.05.08	MD	EDTA	+	-	+		

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
029	Freia	1987	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				n/a		15.05.08	MD	EDTA	+	-	+	-	
				yes		18.11.09	MD	EDTA	+	+	-	+	
030	Robina	1990	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				n/a		25.07.08	MD	EDTA	+	-	+	+	
				yes		18.11.09	MD	EDTA	+	+	-	+	
031	Exotic Lady	1989	M	n/a	O	20.02.08	MD	EDTA	+	-	-	-	
				yes		17.11.09	MD	EDTA	+	+	-	+	
032	Lady Graciana	1997	M	n/a	H	20.02.08	MD	EDTA	+	-	-	-	
				n/a		15.05.08	MD	EDTA	+	-	+	-	
033	Gybsy	1993	M	n/a	n/a	20.02.08	MD	EDTA	-	-	-	-	HM+*, 21.01.08
034	Lady	1988	M	n/a	n/a	20.02.08	MD	EDTA	-	-	-	-	HM+*, 29.11.07
036	Peggy	2007	M	no	H	20.10.08	MD	EDTA	+	-	+	-	suspected HM infection
				no		17.11.09	MD	EDTA	+	+	-	+	NC 3, poor development
042	DeeDee Dakota	n/a	M	n/a	T	20.11.08	MD	EDTA	-	-	+	-	suspected HM infection
043	Grace	n/a	n/a	n/a	n/a	08.12.08	MD	EDTA	+	-	+	-	suspected HM infection
044	n/a	n/a	n/a	n/a	n/a	08.12.08	MD	EDTA	-	-	+	-	suspected HM infection
045	Quite Vanitas	n/a	n/a	n/a	n/a	n/a	MD	EDTA	+	-	+	-	suspected HM infection
046	Reagan	n/a	n/a	n/a	n/a	n/a	MD	-	+	-	-	-	suspected HM infection

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
047	n/a	n/a	n/a	n/a	n/a	n/a	MD	EDTA	+	-	+	-	suspected HM infection
048	Romina S	1997	M	yes	H	16.11.09	MD	EDTA	+	+	-	+	
049	Gesche	1996	M	yes	D	16.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
050	Acordia	1995	M	yes	O	16.11.09	MD	EDTA	+	+	-	+	
051	Belle de Jour	2005	M	no	H	16.11.09	MD	EDTA	+	+	-	+	shaggy, NC 3-4
052	Piccoline	2001	M	yes	W	16.11.09	MD	EDTA	+	+	-	+	
053	Friemo	2001	G	/	H	16.11.09	MD	EDTA	+	+	-	+	lazy, nerveless
054	Luise	2004	M	yes	H	16.11.09	MD	EDTA	+	+	-	+	
055	Dali	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
056	Findus	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
057	Quebec	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
058	Sam	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
059	Lorenz	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
060	Fabian	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
061	Seppi	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
062	Benetton B	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
063	Franz-Josef	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
064	So What	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	AB, infected with *Rh. equi*
						09.12.09	MD	Citrate	-	+	+	+	
065	Catnap	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
066	Sandman	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
067	Montano	2009	S	/	D	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
068	Schneider	2009	S	/	O	17.11.09	MD	EDTA	+	+	-	+	
069	Rocky	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
070	Cosimo	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
071	Squirl	2009	S	/	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
072	Quiddith	2009	S	/	BW	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
073	Dolly	1995	M	no	TH	17.11.09	MD	EDTA	+	+	-	+	
075	Churchill	1996	G	/	B	17.11.09	MD	EDTA	+	+	-	+	
076	Karamé	1995	M	no	T	17.11.09	MD	EDTA	+	+	-	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
077	Kara	2006	M	yes	T	17.11.09	MD	EDTA	+	+	-	+	
078	Absolutely	2003	M	yes	H	17.11.09	MD	EDTA	+	+	-	+	
079	Conny	2006	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
080	Tabora	2006	M	yes	T	17.11.09	MD	EDTA	+	+	-	+	NC recovered
081	Sia	2006	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
082	Donella	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
083	Vitesse	2008	M	no	H	17.11.09	MD	EDTA	+	+	-	+	NC recovered
084	Sahara	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
085	Delight	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
086	Argentina	2003	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
087	Contina	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
088	Pintia	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
089	Clara	2007	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
090	Coralie	2006	M	no	HO	17.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
091	Bretagna	1995	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
092	Gweenie	2006	M	no	H	17.11.09	MD	EDTA	+	+	-	+	
093	Cheyenne		M	no	PI	17.11.09	MD	EDTA	+	+	-	+	
094	Lennert	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
095	Robby	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
096	Paul	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
097	Spock	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
098	Robert	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
099	Winnetou	1998	G	/	TI	18.11.09	MD	EDTA	+	+	-	+	
100	Sita	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
101	Valerie	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
102	Dira	2007	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
103	Dieter	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
104	Keanu	2007	G	/	T	18.11.09	MD	EDTA	+	+	-	+	
105	Mondega	2007	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
106	Lecado	2004	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
107	Vacita	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
108	Cica Trice	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
109	Vicky	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
110	Sentosa	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
111	Katana	2008	M	no	T	18.11.09	MD	EDTA	+	+	-	+	
112	Quirina	2008	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
113	Vita	2007	M	no	H	18.11.09	MD	EDTA	+	+	-	+	
114	Royalty	2007	M	no	H	18.11.09	MD	EDTA	+	+	-	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
115	Ballimo	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
116	Don Absolut	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
117	Basti	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
118	Roberto	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
119	Stanley	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
120	Cicero	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
121	Faro	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
122	Lexo	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
123	Carl-Otto	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
124	Sky Jumper	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
125	Escolit	2007	G	/	H	18.11.09	MD	EDTA	+	+	-	+	
126	Dickens	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
127	Anuk	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
128	Willibald	2008	S	/	H	18.11.09	MD	EDTA	+	+	-	+	
129	Viktor	1983	G	/	T	18.11.09	MD	EDTA	+	+	-	+	
130	Honey Heart	2001	M	no	WU	18.11.09	MD	EDTA	+	+	-	+	
131	Valeca	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
132	Feline	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	-	+	+	+	
133	Elbany	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
134	Selda	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
135	Vally	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	AB because of a lung disease
						09.12.09	MD	Citrate	-	+	+	+	
136	Sissi	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
						09.12.09	MD	Citrate	+	+	+	+	
137	Chiara	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
138	Di	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
139	Verena	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
140	Celestine	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
141	Chandra	2009	M	no	T	19.11.09	MD	EDTA	+	+	-	+	
142	Celina	2009	M	no	H	19.11.09	MD	EDTA	+	+	-	+	
143	Sambucco	n/a	n/a	n/a	n/a	06.01.10	SY	EDTA	-	+	-	+	HM+
144	Quirin	n/a	S	n/a	n/a	06.01.10	SY	EDTA	-	+	-	+	HM+
145	La Bamboula	1994	M	n/a	n/a	29.12.09	SY	EDTA	-	+	-	+	HM+
146	Loretta	n/a	n/a	n/a	n/a	29.12.09	SY	EDTA	-	+	-	+	HM+
147	Notje	2004	n/a	n/a	n/a	16.12.09	SY	EDTA	-	+	-	+	HM+
148	Kanif	2009	S	/	A	30.12.09	SY	EDTA	-	+	-	+	HM+
149	Giuliani	1994	G	/	n/a	10.12.09	SY	EDTA	-	+	-	+	HM+
150	Aragon	2006	G	/	n/a	28.12.09	SY	EDTA	-	+	-	+	HM+
151	Ronja	2003	M	n/a	n/a	17.12.09	SY	EDTA	-	+	-	+	HM+
152	Merlin	n/a	n/a	n/a	n/a	24.01.10	SY	EDTA	-	+	-	+	HM+

Table VIII-1 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
153	Kalle	2005	G	/	SH	19.01.10	SY	EDTA	-	+	-	+	HM+
154	Rebana	n/a	n/a	n/a	n/a	19.01.10	SY	EDTA	-	+	-	+	HM+
155	Cockney	n/a	S	/	n/a	17.01.10	SY	EDTA	-	+	-	+	HM+
156	Silmoor	n/a	G	/	n/a	21.01.10	SY	EDTA	-	+	-	+	HM+
157	Lyn's Halast	n/a	n/a	n/a	n/a	24.01.10	SY	EDTA	-	+	-	+	HM+
158	n/a	n/a	n/a	n/a	n/a	n/a	SY	EDTA	-	+	-	-	HM+
159	n/a	n/a	n/a	n/a	n/a	n/a	SY	EDTA	-	+	-	-	HM+
160	Fury	n/a	n/a	n/a	n/a	n/a	SP	EDTA	-	-	-	-	
161	n/a	n/a	n/a	n/a	n/a	n/a	SY	EDTA	-	+	-	-	

Blood was anti-coagulated with EDTA, Citrate (Vacuette Greiner Bio-One, St. Gallen, CH) or Alsever's Solution (AL); AO = Acridine Orange Staining; GI = Giemsa Staining; SEM = Scanning Electron Microscopy; M = Mare, G = Gelding, S = Stallion; H = Hanoverian, T = Trakehner, F = Friesian, O = Oldenburg, D = German Riding Pony, W = Westphalian, BW = Belgian Warmblood, TH = Thoroughbred, B = Brandenburger, HO = Holsteiner, PI = Pinto, TI = Tinker, WU = Wuerttemberger, A = Arabian, SH = Shetland Pony; MD = Dr. Michael Dieckmann (Equine Practice Beekenhof, GER); SY = samples were sent by synlab.vet (Geesthacht, GER); SP = Sarah Prohaska (IVB, UZH); HM+ means horses were conspicuous in condition and therefore a complete blood count and chemistry was made by the attending veterinarian, HM like particles could be detected in a Giemsa stained peripheral blood smears by synlab.vet (Geesthacht, GER); n/a = not available; NC = nutritional condition (according to the guidelines of the American Association of Equine Practitioners (AAEP): 1 = poor, 2 = very thin, 3 = thin, 4 = moderately thin, 5 = moderate (optimal condition), 6 = moderate to fleshy, 7 = fleshy, 8 = fat, 9 = extremely fat); AB = treated with antibiotics; *Rh. equi* = *Rhodococcus equi*.

Table VIII-2 Negative horse blood samples collected during this study.

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
035	Rivano	n/a	G	/	n/a	31.07.08	LF	EDTA	+	-	+	-	
037	Joya	1998	M	n/a	Q	10.11.08	CH	EDTA	+	-	+	-	
038	Fiona	2002	M	n/a	FR	10.11.08	CH	EDTA	+	-	+	-	
039	Vivaldi	1999	n/a	/	n/a	10.11.08	CH	EDTA	+	-	+	-	
040	Casper	2002	n/a	/	n/a	10.11.08	CH	EDTA	+	-	+	-	
041	Da Vinci VI	1996	n/a	/	n/a	10.11.08	CH	EDTA	+	-	+	-	
164	Falun	1997	G/S	n/a	W	15.07.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
165	n/a	n/a	n/a	n/a	I	n/a	RH	EDTA	-	-	-	+	negative for any HM (305)*
166	Schiwago	1988	G/S	/	P	27.07.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
167	Charmeuse	1981	M	n/a	FR	30.07.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
168	Igor	1990	G/S	/	DU	05.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
169	Bavaria	2009	M	n/a	SW	06.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
170	Fjallar	1994	G/S	/	I	08.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
171	Taktur	1991	G/S	/	I	10.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
172	Raziella	2004	M	n/a	SW	10.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
173	n/a	2009	S	/	WC	10.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
174	Carla	2000	M	n/a	n/a	10.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
175	Jimy	2002	G/S	/	MA	11.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
176	Adino II	1994	G/S	/	SW	12.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*

Table VIII-2 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
177	Magic Merlin	2004	M	n/a	IR	12.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
178	Gustur	2009	S	/	I	18.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
179	Kivien Crown	1998	G/S	/	ST	17.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
180	Cupido	2000	G/S	/	SH	20.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
181	Lona	1970	M	n/a	P	20.08.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
182	Wisky	2001	G/S	/	TI	07.09.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
183	Svipa	2009	M	n/a	I	08.09.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
184	Terry	1986	M	n/a	SN	16.09.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
185	Carmien	1990	M	n/a	SF	22.09.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
186	Picolo	1990	G/S	/	SH	23.09.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
187	Ecki	2006	G/S	/	SX	05.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
188	Nikita	2001	M	n/a	ST	08.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
189	Fabio	2004	G/S	/	WC	09.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
190	Falina	2004	M	n/a	TH	12.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
191	Andaluz	2000	G/S	/	n/a	12.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
192	Targa	2001	M	n/a	Q	12.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
193	Ramzes B	1999	G/S	/	SW	13.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
194	Hamilton Darcy	1988	G/S	/	IR	13.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
195	Gitan	1985	G/S	/	SH	13.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
196	Snake River	2007	M	n/a	TH	13.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
197	Tigre	1984	G/S	/	DP	14.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*

Table VIII-2 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
198	Lazar	1996	G/S	/	B	14.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
199	Stjarni	1989	G/S	/	I	15.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
200	Amadeus	1998	G/S	/	IR	15.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
201	Nevada	1993	M	n/a	DU	16.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
202	Baroness Senta	1993	M	n/a	HA	16.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
203	Maisi	1989	M	n/a	IT	16.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
204	Montan	2001	G/S	/	ME	20.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
205	Una	2002	M	n/a	FR	20.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
206	Bolestro	1988	G/S	/	H	20.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
207	Carmencita	1999	M	n/a	Q	20.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
208	Pantau	2002	G/S	/	P	21.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
209	Baxte	2002	G/S	/	CZ	21.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
210	Isar	1990	G/S	/	SW	22.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
211	Danceur	1990	G/S	/	PE	22.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
212	Heya	2004	M	n/a	FR	22.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
213	Ricky	2004	G/S	/	Q	23.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
214	Jonny-Boy	1984	G/S	/	I	24.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
215	Queen	1991	M	n/a	TH	26.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
216	Angel Bea Doc	1984	M	n/a	Q	26.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
217	Romeo	1999	G/S	/	HU	26.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
218	Branca	1993	M	n/a	N	26.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*

Table VIII-2 continued

No.	Horse	Age	Gender	Pregnancy	Breed	Sampling Date	Sampled by	Blood	Peripheral blood smear		SEM preparation	Complete blood count	Preliminary report
									AO	GI			
219	Patches	2002	M	n/a	PH	27.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
220	Granissa	1997	M	n/a	O	27.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
221	Nando	1996	G/S	/	T	28.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
222	Lagano	1993	G/S	/	HO	29.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
223	Pablo	2003	G/S	/	SH	31.10.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
224	Lorenzo	2006	G/S	/	SC	02.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
225	Agogo	2005	M	n/a	SW	03.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
226	Odin	2003	G/S	/	I	03.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
227	Nico	1995	G/S	/	HA	03.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
228	Corentilla	2006	M	n/a	SW	03.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*
229	Hero	1991	G/S	/	BW	04.11.09	RH	EDTA	-	-	-	+	negative for any HM (305)*

Legend see Table VII-1; G/S = desexed status unknown; Q = Quarter Horse, FR = Freiberger, I = Icelandic Horse, P = Pony, DU = Dutch Warmblood, SW = Swiss Warmblood, WC = Welsh Cob Pony, MA = Macedonian Warmblood, IR = Irish Sport Horse, ST = Standardbred, SN = Spanish Warmblood, SF = Selle Francaise, SX = Saxon Warmblood, DP = Dutch Pony, HA = Haflinger, IT = Sella Italiano, ME = Mecklenburger, CZ = Czech Warmblood, PE = Percheron, HU = Hungarian Warmblood, N = Noriker, PH = Paint Horse; SC = Swedish Warmblood, LF = Larissa Fuchs (IVB, UZH), CH = Chirurgical department, Vetclinics, UZH, RH = blood samples were provided by Regina Hofmann-Lehmann, Vetclinics, UZH; * samples were checked by a SYBR green I PCR assay comprising all known HM (366).

VIII.3 Summary of complete blood counts

Table VIII-3 Complete blood counts of samples collected in Autumn/ Winter 2009/ 2010. Abnormal values are highlighted in colour. Repeated sampling of 24 samples in December 2009 is highlighted in yellow (compare Table VIII-1, pp. 165-175). Blood counts were produced by synlab.vet (Geesthacht, GER).

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
001	Dacana	7.2	8.80	137	0.37	41.7	15.6	37.3	113	54	3888	0	0	37	2664	5	360	3	216	1	72	0	0
002	Shirley	6.9	8.28	138	0.38	46.3	16.7	36.0	188	58	4002	0	0	35	2415	5	345	2	138	0	0	0	0
003	Bretagne	7.7	6.54	115	0.33	50.0	17.6	35.2	232	58	4466	0	0	34	2618	4	308	3	231	1	77	0	0
003	Bretagne	7.3	6.61	117	0.33	49.8	17.7	35.6	182	61	4453	0	0	31	2263	4	292	3	219	1	73	0	0
004	Weltfee	10.1	8.22	133	0.36	43.6	16.2	37.2	208	66	6666	0	0	23	2323	5	505	6	606	0	0	0	0
005	Emelie	5.8	8.87	140	0.39	43.6	15.8	36.2	120	53	3074	0	0	37	2146	5	290	4	232	1	58	0	0
006	Leca	8.0	8.91	141	0.40	44.4	15.8	35.6	222	49	3920	0	0	41	3280	4	320	5	400	1	80	0	0
007	Royal Star	7.9	8.84	139	0.38	43.2	15.7	36.4	162	48	3792	0	0	42	3318	4	316	5	395	1	79	0	0
010	Dipsy	6.0	8.29	118	0.32	38.1	14.2	37.3	159	54	3240	0	0	35	2100	6	360	3	180	2	120	0	0
011	Kaja	7.0	7.60	122	0.35	45.7	16.1	35.2	169	56	3920	0	0	36	2520	5	350	2	140	1	70	0	0
012	Darcy	7.3	6.85	115	0.33	48.0	16.8	35.0	212	63	4599	0	0	28	2044	6	438	1	73	2	146	0	0
014	Kassiopeia	5.3	7.20	129	0.36	49.6	17.9	36.1	108	56	2968	0	0	37	1961	3	159	3	159	1	53	0	0
015	Wolkenfee	7.4	7.38	124	0.35	47.2	16.8	35.6	196	60	4440	0	0	34	2516	5	370	1	74	0	0	0	0
015	Wolkenfee	7.0	6.98	118	0.33	47.3	16.9	35.8	201	58	4060	0	0	37	2590	4	280	1	70	0	0	0	0
020	Escot	8.3	7.64	115	0.31	41.1	15.1	36.6	121	43	3569	0	0	49	4067	4	332	3	249	1	83	0	0
020	Escot	7.1	7.67	114	0.31	40.7	14.9	36.5	111	46	3266	0	0	48	3408	4	284	2	142	0	0	0	0
021	Lilli	11.0	8.54	143	0.39	46.0	16.7	36.4	157	26	2860	0	0	70	7700	0	0	4	440	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
022	Dondra	8.3	9.00	133	0.37	40.7	14.8	36.3	125	53	4399	0	0	36	2988	6	498	4	332	1	83	0	0
023	Dorit	9.8	9.22	139	0.37	39.6	15.1	38.1	153	54	5292	0	0	38	3724	5	490	2	196	1	98	0	0
024	Tristan	7.6	6.56	112	0.33	49.8	17.1	34.3	196	51	3876	0	0	40	3040	5	380	3	228	1	76	0	0
025	Caray	6.8	9.27	140	0.39	41.6	15.1	36.3	164	53	3604	0	0	38	2584	5	340	4	272	0	0	0	0
026	Rubina	9.7	8.39	142	0.36	43.4	16.9	39.0	122	50	4850	0	0	38	3686	4	388	7	679	1	97	0	0
029	Freia	8.1	7.42	129	0.36	48.8	17.4	35.6	146	55	4455	0	0	36	2916	3	243	6	486	0	0	0	0
030	Robina	8.3	8.36	147	0.41	49.3	17.6	35.7	107	56	4648	0	0	34	2822	5	415	5	415	0	0	0	0
031	Exotic Lady	8.0	8.35	138	0.42	50.7	16.5	32.6	149	52	4160	0	0	43	3440	3	240	2	160	0	0	0	0
036	Peggy	10.8	8.94	124	0.33	36.7	13.9	37.8	140	47	5076	0	0	44	4752	5	540	3	324	1	108	0	0
048	Romina S	8.2	8.84	132	0.36	40.3	14.9	37.1	165	59	4838	0	0	30	2460	4	328	6	492	1	82	0	0
049	Gesche	7.2	8.54	141	0.39	45.4	16.5	36.3	189	47	3384	0	0	45	3240	4	288	3	216	1	72	0	0
049	Gesche	6.8	8.62	142	0.39	45.7	16.5	36.0	170	42	2856	0	0	50	3400	5	340	2	136	1	68	0	0
050	Acordia	7.4	8.37	141	0.39	46.5	16.8	36.2	180	57	4218	0	0	33	2442	6	444	3	222	1	74	0	0
051	Belle de Jour	11.5	8.62	141	0.38	43.6	16.4	37.5	134	35	4025	0	0	61	7015	3	345	1	115	0	0	0	0
052	Piccoline	7.8	7.25	126	0.37	50.8	17.4	34.2	149	54	4212	0	0	36	2808	4	312	5	390	1	78	0	0
053	Friemo	7.5	7.46	127	0.35	46.8	17.0	36.4	104	46	3450	0	0	45	3375	3	225	3	225	3	225	0	0
054	Luise	8.7	9.12	144	0.38	41.9	15.8	37.7	176	44	3828	0	0	49	4263	4	348	2	174	1	87	0	0
055	Dali	9.3	7.91	110	0.30	38.3	13.9	36.3	196	50	4650	0	0	41	3813	6	558	3	279	0	0	0	0
056	Findus	10.3	9.90	118	0.31	30.9	11.9	38.6	301	45	4635	0	0	48	4944	6	618	1	103	0	0	0	0
057	Quebec	11.4	7.09	94	0.26	36.8	13.3	36.0	234	50	5700	0	0	46	5244	4	456	0	0	0	0	0	0
057	Quebec	15.0	8.30	112	0.31	37.7	13.5	35.8	126	38	5700	0	0	56	8400	2	300	4	600	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
058	Sam	9.8	9.55	120	0.32	33.4	12.6	37.6	220	34	3332	0	0	63	6174	0	0	3	294	0	0	0	0
059	Lorenz	11.1	8.28	109	0.29	34.7	13.2	38.0	183	52	5772	0	0	44	4884	4	444	0	0	0	0	0	0
059	Lorenz	16.7	7.94	105	0.28	35.1	13.2	37.6	174	62	10354	0	0	38	6346	0	0	0	0	0	0	0	0
060	Fabian	13.8	9.55	120	0.31	32.7	12.6	38.5	220	32	4416	0	0	66	9108	2	276	0	0	0	0	0	0
060	Fabian	16.3	10.05	126	0.34	33.4	12.5	37.5	243	54	8802	0	0	46	7498	0	0	0	0	0	0	0	0
061	Seppi	9.8	9.91	123	0.31	31.6	12.4	39.3	145	42	4116	0	0	56	5488	0	0	2	196	0	0	0	0
062	Benetton B	7.6	7.58	100	0.28	36.8	13.2	35.8	170	31	2356	0	0	60	4560	6	456	3	228	0	0	0	0
062	Benetton B	12.2	9.58	126	0.34	35.9	13.2	36.6	198	36	4392	0	0	60	7320	4	488	0	0	0	0	0	0
063	Franz-Josef	9.6	9.53	114	0.31	32.0	12.0	37.4	250	34	3264	0	0	60	5760	2	192	4	384	0	0	0	0
063	Franz-Josef	13.4	10.85	130	0.35	31.8	12.0	37.7	280	48	6432	0	0	52	6968	0	0	0	0	0	0	0	0
064	So What	11.7	8.86	112	0.30	33.4	12.6	37.8	245	42	4914	0	0	54	6318	2	234	0	0	2	234	0	0
064	So What	17.0	9.04	117	0.32	35.1	12.9	36.9	236	60	10200	0	0	40	6800	0	0	0	0	0	0	0	0
065	Catnap	10.3	10.25	123	0.32	31.6	12.0	38.0	288	45	4635	0	0	46	4738	6	618	3	309	0	0	0	0
066	Sandman	13.1	9.04	112	0.30	32.9	12.4	37.7	103	28	3668	0	0	68	8908	2	262	2	262	0	0	0	0
067	Montano	11.5	9.03	112	0.31	33.8	12.4	36.7	286	32	3680	0	0	66	7590	2	230	0	0	0	0	0	0
067	Montano	12.9	9.47	120	0.33	34.3	12.7	36.9	246	32	4128	0	0	68	8772	0	0	0	0	0	0	0	0
068	Schneider	10.0	9.31	122	0.33	35.3	13.1	37.1	249	52	5200	0	0	44	4400	4	400	0	0	0	0	0	0
069	Rocky	9.2	7.93	109	0.30	38.0	13.7	36.2	171	41	3772	0	0	50	4600	5	460	4	368	0	0	0	0
069	Rocky	9.1	8.45	116	0.32	38.2	13.7	35.9	123	32	2912	0	0	68	6188	0	0	0	0	0	0	0	0
070	Cosimo	8.6	7.72	104	0.28	36.7	13.5	36.7	184	35	3010	0	0	57	4902	6	516	2	172	0	0	0	0
070	Cosimo	11.5	9.84	130	0.35	35.7	13.2	37.0	206	26	2990	0	0	74	8510	0	0	0	0	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
071	Squirl	8.1	8.43	111	0.30	35.9	13.2	36.6	118	41	3321	0	0	48	3888	7	567	3	243	1	81	0	0
071	Squirl	12.6	8.16	106	0.30	36.4	13.0	35.7	174	58	7308	0	0	37	4662	4	504	1	126	0	0	0	0
072	Quiddith	14.3	8.95	111	0.29	32.8	12.4	37.8	171	40	5720	0	0	56	8008	4	572	0	0	0	0	0	0
072	Quiddith	19.5	9.57	119	0.32	33.2	12.4	37.4	149	28	5460	0	0	70	13650	2	390	0	0	0	0	0	0
073	Dolly	6.6	8.07	122	0.33	40.9	15.1	37.0	172	49	3234	0	0	42	2772	5	330	3	198	1	66	0	0
075	Churchill	6.2	8.50	133	0.37	43.1	15.6	36.3	104	63	3906	0	0	28	1736	5	310	3	186	1	62	0	0
076	Karamé	7.1	7.86	128	0.35	45.0	16.3	36.2	149	60	4260	0	0	31	2201	4	284	5	355	0	0	0	0
077	Kara	10.6	8.71	135	0.37	42.6	15.5	36.4	100	50	5300	0	0	43	4558	4	424	3	318	0	0	0	0
078	Absolutely	7.7	8.42	141	0.38	45.5	16.7	36.8	234	60	4620	0	0	30	2310	4	308	5	385	1	77	0	0
079	Conny	8.9	9.02	143	0.37	41.0	15.9	38.6	144	48	4272	0	0	44	3916	4	356	2	178	2	178	0	0
080	Tabora	10.2	8.68	137	0.39	44.4	15.8	35.6	136	46	4692	0	0	47	4794	3	306	3	306	1	102	0	0
081	Sia	9.3	8.91	133	0.36	40.6	14.9	36.7	110	46	4278	0	0	49	4557	4	372	1	93	0	0	0	0
082	Donella	7.1	8.92	123	0.32	36.2	13.8	38.1	171	53	3763	0	0	37	2627	4	284	5	355	1	71	0	0
083	Vitesse	11.1	9.22	131	0.34	37.0	14.2	38.4	136	30	3330	0	0	66	7326	4	444	0	0	0	0	0	0
084	Sahara	12.5	9.58	132	0.36	37.1	13.8	37.2	91	51	6375	0	0	46	5750	0	0	2	250	1	125	0	0
085	Delight	11.1	8.93	131	0.35	39.6	14.7	37.0	272	50	5550	0	0	44	4884	3	333	2	222	1	111	0	0
085	Delight	9.4	8.27	122	0.33	39.9	14.8	37.0	177	45	4230	0	0	47	4418	4	376	3	282	1	94	0	0
086	Argentina	7.2	7.53	115	0.31	41.7	15.3	36.6	165	52	3744	0	0	41	2952	4	288	2	144	1	72	0	0
087	Contina	10.6	9.28	128	0.33	35.8	13.8	38.6	144	43	4558	0	0	45	4770	4	424	7	742	1	106	0	0
088	Pintia	12.8	9.11	132	0.34	37.2	14.5	38.9	214	39	4992	0	0	57	7296	1	128	3	384	0	0	0	0
089	Clara	14.1	9.48	131	0.34	36.0	13.8	38.4	221	24	3384	0	0	70	9870	2	282	0	0	4	564	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
090	Coralie	8.3	7.62	125	0.34	44.2	16.4	37.1	140	46	3818	0	0	44	3652	5	415	3	249	2	166	0	0
090	Coralie	6.4	6.82	111	0.31	45.0	16.3	36.2	148	41	2624	0	0	50	3200	7	448	1	64	1	64	0	0
091	Bretagna	7.5	8.09	140	0.37	45.6	17.3	37.9	119	52	3900	0	0	41	3075	4	300	2	150	1	75	0	0
092	Gweenie	9.3	7.50	115	0.31	41.9	15.3	36.6	128	47	4371	0	0	46	4278	4	372	2	186	1	93	0	0
093	Cheyenne	10.5	8.49	152	0.41	48.5	17.9	36.9	121	65	6825	0	0	28	2940	4	420	3	315	0	0	0	0
094	Lennert	3.6	13.38	212	0.52	38.7	15.8	40.9	83	37	1332	0	0	54	1944	6	216	2	72	1	36	0	0
095	Robby	7.7	8.07	117	0.32	39.0	14.5	37.1	154	40	3080	0	0	48	3696	7	539	4	308	1	77	0	0
096	Paul	10.8	8.89	128	0.34	37.9	14.4	38.0	118	26	2808	0	0	70	7560	2	216	0	0	2	216	0	0
097	Spock	9.9	10.26	154	0.38	36.6	15.0	41.0	189	55	5445	0	0	36	3564	5	495	3	297	1	99	0	0
098	Robert	11.1	7.83	120	0.32	41.3	15.3	37.2	135	57	6327	0	0	32	3552	6	666	4	444	1	111	0	0
098	Robert	9.2	8.99	135	0.36	40.3	15.0	37.3	150	54	4968	0	0	33	3036	8	736	3	276	2	184	0	0
099	Winnetou	8.1	6.87	121	0.34	49.8	17.6	35.4	143	50	4050	0	0	38	3078	6	486	6	486	0	0	0	0
100	Sita	10.0	9.73	137	0.36	36.9	14.1	38.2	201	42	4200	0	0	50	5000	5	500	3	300	0	0	0	0
101	Valerie	9.6	9.88	140	0.37	36.9	14.2	38.4	221	44	4224	0	0	47	4512	4	384	4	384	1	96	0	0
102	Dira	13.5	8.98	126	0.33	36.5	14.0	38.4	199	28	3780	0	0	62	8370	6	810	4	540	0	0	0	0
103	Dieter	9.2	7.90	124	0.34	42.4	15.7	37.0	159	46	4232	0	0	44	4048	6	552	3	276	1	92	0	0
103	Dieter	8.7	7.87	123	0.34	43.3	15.6	36.1	123	42	3654	0	0	42	3654	10	870	5	435	1	87	0	0
104	Keanu	10.3	8.80	137	0.39	43.8	15.6	35.6	113	49	5047	0	0	42	4326	4	412	4	412	1	103	0	0
105	Mondega	10.7	8.42	116	0.31	36.8	13.8	37.4	174	48	5136	0	0	43	4601	5	535	3	321	1	107	0	0
106	Lecado	5.9	7.99	121	0.34	42.4	15.1	35.7	175	47	2773	0	0	46	2714	3	177	4	236	0	0	0	0
107	Vacita	11.7	9.56	123	0.32	33.7	12.9	38.2	185	38	4446	0	0	58	6786	4	468	0	0	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
108	Cica Trice	10.7	9.66	132	0.35	36.0	13.7	37.9	129	32	3424	0	0	64	6848	4	428	0	0	0	0	0	0
109	Vicky	7.4	8.82	120	0.33	37.5	13.6	36.3	195	51	3774	0	0	41	3034	5	370	2	148	1	74	0	0
110	Sentosa	8.6	8.54	123	0.32	37.9	14.4	38.0	165	51	4386	0	0	40	3440	5	430	3	258	1	86	0	0
111	Katana	6.4	9.14	127	0.33	35.7	13.9	39.0	152	64	4096	0	0	28	1792	5	320	2	128	1	64	0	0
112	Quirina	8.5	8.40	127	0.34	40.1	15.1	37.7	166	56	4760	0	0	35	2975	5	425	3	255	1	85	0	0
113	Vita	9.3	9.20	142	0.38	40.9	15.4	37.8	130	42	3906	0	0	49	4557	4	372	4	372	1	93	0	0
114	Royalty	11.9	9.27	150	0.40	42.7	16.2	37.9	129	38	4522	0	0	54	6426	6	714	2	238	0	0	0	0
115	Ballimo	10.6	9.99	136	0.36	36.3	13.6	37.5	154	53	5618	0	0	37	3922	6	636	3	318	1	106	0	0
116	Don Absolut	9.7	10.32	141	0.37	36.1	13.7	37.8	236	48	4656	0	0	43	4171	5	485	3	291	1	97	0	0
117	Basti	11.3	9.31	133	0.35	37.8	14.3	37.8	123	57	6441	0	0	33	3729	6	678	3	339	1	113	0	0
118	Roberto	10.3	8.28	120	0.32	38.0	14.5	38.1	190	46	4738	0	0	42	4326	7	721	4	412	1	103	0	0
118	Roberto	11.3	8.10	117	0.30	37.4	14.4	38.6	226	50	5650	0	0	40	4520	6	678	4	452	0	0	0	0
119	Stanley	11.9	10.11	138	0.35	34.5	13.6	39.5	225	52	6188	0	0	39	4641	5	595	4	476	0	0	0	0
120	Cicero	10.0	9.89	136	0.35	35.7	13.8	38.5	149	45	4500	0	0	43	4300	5	500	6	600	1	100	0	0
121	Faro	13.0	8.44	130	0.34	40.4	15.4	38.1	128	24	3120	0	0	68	8840	2	260	4	520	2	260	0	0
122	Lexo	12.3	9.24	137	0.37	39.9	14.8	37.1	146	24	2952	0	0	74	9102	2	246	0	0	0	0	0	0
123	Carl-Otto	10.9	9.83	131	0.35	35.2	13.3	37.9	185	52	5668	0	0	41	4469	4	436	3	327	0	0	0	0
124	Sky Jumper	11.4	10.13	141	0.36	35.3	13.9	39.4	179	50	5700	0	0	48	5472	2	228	0	0	0	0	0	0
125	Escolit	11.5	8.87	132	0.35	39.3	14.9	37.8	121	38	4370	0	0	56	6440	2	230	4	460	0	0	0	0
126	Dickens	12.7	8.87	130	0.34	38.8	14.7	37.8	195	42	5334	0	0	50	6350	2	254	4	508	2	254	0	0
127	Anuk	12.6	8.86	120	0.32	36.3	13.5	37.3	144	32	4032	0	0	60	7560	2	252	6	756	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
128	Willibald	13.0	11.75	154	0.39	33.4	13.1	39.3	242	52	6760	0	0	38	4940	5	650	4	520	1	130	0	0
129	Viktor	6.6	8.12	140	0.38	46.9	17.2	36.7	129	63	4158	0	0	29	1914	4	264	4	264	0	0	0	0
130	Honey Heart	5.8	8.55	136	0.36	41.6	15.9	38.2	109	53	3074	0	0	37	2146	6	348	4	232	0	0	0	0
131	Valeca	10.9	8.76	108	0.29	33.3	12.3	37.0	162	34	3706	0	0	60	6540	4	436	2	218	0	0	0	0
131	Valeca	10.7	9.64	120	0.32	32.8	12.4	38.0	190	28	2996	0	0	72	7704	0	0	0	0	0	0	0	0
132	Feline	12.2	8.57	112	0.29	34.3	13.1	38.1	160	20	2440	0	0	74	9028	6	732	0	0	0	0	0	0
132	Feline	12.7	9.60	126	0.33	34.2	13.1	38.4	117	38	4826	0	0	62	7874	0	0	0	0	0	0	0	0
133	Elbany	12.3	10.64	126	0.33	31.1	11.8	38.1	121	44	5412	0	0	52	6396	4	492	0	0	0	0	0	0
134	Selda	11.2	10.65	131	0.35	32.5	12.3	37.9	201	28	3136	0	0	66	7392	4	448	2	224	0	0	0	0
135	Vally	10.8	8.34	106	0.29	34.3	12.7	37.1	183	54	5832	0	0	37	3996	7	756	1	108	1	108	0	0
135	Vally	13.8	8.25	107	0.30	35.8	13.0	36.3	188	68	9384	0	0	30	4140	2	276	0	0	0	0	0	0
136	Sissi	13.2	9.52	123	0.33	34.6	12.9	37.4	320	38	5016	0	0	52	6864	4	528	4	528	2	264	0	0
136	Sissi	15.1	9.49	125	0.34	36.1	13.2	36.4	330	52	7852	0	0	48	7248	0	0	0	0	0	0	0	0
137	Chiara	11.3	10.29	129	0.34	32.7	12.5	38.4	142	50	5650	0	0	48	5424	2	226	0	0	0	0	0	0
138	Di	11.5	9.75	124	0.33	33.6	12.7	37.8	309	47	5405	0	0	43	4945	7	805	3	345	0	0	0	0
139	Verena	14.0	9.02	116	0.32	35.0	12.9	36.7	272	60	8400	0	0	32	4480	8	1120	0	0	0	0	0	0
140	Celestine	7.5	9.15	119	0.32	34.4	13.0	37.8	127	45	3375	0	0	46	3450	6	450	2	150	1	75	0	0
141	Chandra	10.7	9.15	119	0.31	34.0	13.0	38.3	228	43	4601	0	0	45	4815	6	642	5	535	1	107	0	0
142	Celina	8.7	9.50	121	0.32	33.3	12.7	38.3	220	30	2610	0	0	64	5568	4	348	2	174	0	0	0	0
143	Sambucco	6.1	7.61	125	0.35	46.0	16.4	35.7	100	50	3050	0	0	40	2440	8	488	1	61	1	61	0	0
144	Qurin	6.6	6.75	115	0.32	47.3	17.0	36.1	158	50	3300	0	0	35	2310	6	396	9	594	0	0	0	0

Table VIII-3 continued

		Complete blood count								Differential haemogram													
No.	**Identification**	**Leucocytes**	**Erythrocytes**	**Haemoglobin**	**Haematocrit**	**MCV**	**MCH**	**MCHC**	**Thrombocytes**	**Segmented Neutrophils**	**Segmented Neutrophils abs.**	**Band Neutrophils**	**Band Neutrophils abs.**	**Lymphocytes**	**Lymphocytes abs.**	**Monocytes**	**Monocytes abs.**	**Eosinophils**	**Eosinophils abs.**	**Basophils**	**Basophils abs.**	**Other cells**	**Other cells abs.**
Reference range		6.0-12.0 G/l	6.0-12.0 T/l	100-180 g/l	0.32-0.48 l/l	34-58 fl	13-19 pg	31-37 g/dl	100-600 G/l	30-75 %	3000-6000 /µl	0-1 %	0-100 /µl	25-60 %	1500-5000 /µl	1-8 %	0-100 /µl	1-10 %	0-800 /µl	0-3 %	0-300 /µl	0 %	/µl
145	La Bamboula	8.5	3.00	53	0.15	51.0	17.7	34.6	72	83	7055	0	0	9	765	8	680	0	0	0	0	0	0
146	Loretta	5.1	5.00	91	0.25	50.4	18.2	36.1	143	43	2193	0	0	50	2550	4	204	3	153	0	0	0	0
147	Notje	8.5	6.11	98	0.26	42.1	16.0	38.1	/	45	3825	0	0	50	4250	3	255	1	85	1	85	0	0
148	Kanif	14.3	8.62	101	0.28	32.6	11.7	35.9	241	36	5148	0	0	60	8580	4	572	0	0	0	0	0	0
149	Giuliani	13.3	6.83	114	0.32	46.3	16.7	36.1	130	79	10507	0	0	21	2793	0	0	0	0	0	0	0	0
150	Aragon	30.4	6.88	105	0.29	41.6	15.3	36.7	332	83	25232	0	0	14	4256	1	304	2	608	0	0	0	0
151	Ronja	5.5	5.80	105	0.29	50.0	18.1	36.2	195	28	1540	?	?	72	3960	0	0	0	0	0	0	0	0
152	Merlin	2.6	6.57	115	0.31	47.6	17.5	36.7	121	50	1300	0	0	44	1144	6	156	0	0	0	0	0	0
153	Kalle	10.2	7.52	118	0.33	43.2	15.7	36.3	115	34	3468	?	?	62	6324	0	0	4	408	0	0	0	0
154	Rebana	6.4	7.84	132	0.40	51.3	16.8	32.8	101	59	3776	0	0	34	2176	5	320	2	128	0	0	0	0
155	Cockney	6.0	6.14	94	0.27	44.1	15.3	34.7	115	56	3360	0	0	39	2340	4	240	1	60	0	0	0	0
156	Silmoor	4.6	5.75	96	0.27	47.5	16.7	35.2	112	55	2530	?	?	37	1702	6	276	2	92	0	0	0	0
157	Lyn's Halast	19.6	4.90	69	0.20	40.0	14.1	35.2	161	74	14504	0	0	18	3528	8	1568	0	0	0	0	0	0

No. = samples number; MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration; blue = decreased, orange = increased; yellow = re-sampled horses; / = not determined.

VIII.4 Sequence alignments

On the following pages the corresponding alignments of the phylogenetic trees shown in Chapter IV will be presented.

Annotation of sequences is according to the IUPAC nomenclature: A = Adenine, C = Cytosine, G = Guanine, T (or U) = Thymine (or Uracil), R = A or G, Y = C or T, S = G or C, W = A or T (U), K = G or T (U), M = A or C, B = C or G or T (U), D = A or G or T (U), H = A or C or T (U), V = A or C or G, N = any base, . or - = gap.

Sequence position is given on the top, on the left and on the right.

On the left side, species are assigned by an 'ARB short name'. For explanation of abbreviations see Table VIII-4.

Table VIII-4 Description of the 16S rRNA isolates used in the phylogenetic studies

ARB	Accession	Description	Host	bp
BacSubti	AB042061	*Bacillus subtilis*		1550
BacSub76	Z99107	*Bacillus subtilis*		1550
CloPe117	CP000246	*Clostridium perfringens*		1518
CanMy155	AB558897	*C*M erythrocervae	*Cervus nippon*	1434
CanMy157	AB558898	*C*M erythrocervae	*Cervus nippon*	1434
MycEryth	AF178676	*C*M erythrodidelphis	*Didelphis marsupialis*	1453
CanMyc10	AY383241	*C*M haematoparvum	Dog	1461
CanMyc12	AY532390	*C*M haematoparvum	Dog	1448
CanMyc50	EF416569	*C*M haematoparvum	Dog	1351
CanMy131	GQ129112	*C*M haematoparvum	Dog	1310
CanMy132	GQ129114	*C*M haematoparvum	Dog	1310
CanMy137	GQ129113	*C*M haematoparvum	Dog	1310
CanMyc51	EF460765	*C*M haemobovis	Cattle	1435
CanMyc52	EF616467	*C*M haemobovis	Cattle	1391
CanMyc53	EF616468	*C*M haemobovis	Cattle	1391
CanMyc59	EU367965	*C*M haemobovis	Cattle	1204
CanMy156	AB558899	*C*M haemocervae	*Cervus nippon*	1434

Table VIII-4 continued

ARB	Accession	Description	Host	bp
MycHaemo	AF306346	*C*M haemolamae	Alpaca	1428
CanMy122	FN908076	*C*M haemolamae	Llama	1080
CanMy123	FN908077	*C*M haemolamae	Llama	1071
CanMy124	FN908078	*C*M haemolamae	Llama	1075
CanMy125	FN908079	*C*M haemolamae	Llama	1078
CanMy126	FN908080	*C*M haemolamae	Llama	1070
CanMy127	FN908081	*C*M haemolamae	Llama	1032
CanMy128	FN908082	*C*M haemolamae	Llama	1068
CanMy129	FN908083	*C*M haemolamae	Llama	1080
CanMy130	FN908084	*C*M haemolamae	Llama	1065
CanMy153	GU047355	*C*M haemolamae	Alpaca	1421
CanMycop	AF271154	*C*M haemominutum	Cat	1425
CanMyco3	AM745338	*C*M haemominutum	Cat	1456
CanMyco4	AY150974	*C*M haemominutum	Cat	1426
CanMyco6	AY150979	*C*M haemominutum	Cat	1427
CanMyco7	AY150980	*C*M haemominutum	Cat	1425
CanMyco8	AY150981	*C*M haemominutum	Cat	1424
CanMyco9	AY297712	*C*M haemominutum	Dog	1480
CanMyc11	AY529634	*C*M haemominutum	Cat	1291
CanMyc13	DQ157141	*C*M haemominutum	Cat	1356
CanMyc14	DQ157143	*C*M haemominutum	Cat	1359
CanMyc16	DQ157145	*C*M haemominutum	Cat	1356
CanMyc17	DQ157146	*C*M haemominutum	Cat	1358
CanMyc18	DQ157147	*C*M haemominutum	Cat	1356
CanMyc19	DQ157148	*C*M haemominutum	Cat	1359
CanMyc20	DQ157149	*C*M haemominutum	Cat	1356
CanMyc35	DQ825439	*C*M haemominutum	*Leopardus tigrinus*	1355
CanMyc36	DQ825440	*C*M haemominutum	*Leopardus wiedii*	1352
CanMyc37	DQ825442	*C*M haemominutum	European wildcat	1358
CanMyc38	DQ825443	*C*M haemominutum	European wildcat	1355
CanMyc39	DQ825444	*C*M haemominutum	Iberian lynx	1357
CanMyc40	DQ825445	*C*M haemominutum	Iberian lynx	1358
CanMyc41	DQ825446	*C*M haemominutum	Iberian lynx	1360
CanMyc45	DQ825452	*C*M haemominutum	Lion	1356
CanMyc47	DQ825455	*C*M haemominutum	Lion	1356
CanMyc48	DQ825456	*C*M haemominutum	Eurasian lynx	1366
CanMyc49	DQ825457	*C*M haemominutum	Eurasian lynx	1359
CanMyc54	EU128752	*C*M haemominutum	Cat	1274
CanMyc57	EU170604	*C*M haemominutum	*Ctenocephalides felis*	1268
CanMyc64	EU839979	*C*M haemominutum	*Felis catus*	1257
CanMyc65	EU839980	*C*M haemominutum	*Felis catus*	1305
CanMyc66	EU839981	*C*M haemominutum	*Felis catus*	1305
CanMyc67	EU839982	*C*M haemominutum	*Felis catus*	1305
CanMyc68	EU839983	*C*M haemominutum	*Felis catus*	1306
CanMyc69	EU839984	*C*M haemominutum	*Felis catus*	1306
CanMyc70	EU839985	*C*M haemominutum	*Felis catus*	1306

Table VIII-4 continued

ARB	Accession	Description	Host	bp
CanMyc72	FJ004275	*C*M haemominutum	*Felis catus*	1312
CanMyc73	U88564	*C*M haemominutum	Cat	1430
CanMy136	AM691834	*C*M haemominutum	Dog	1457
CanMyc55	EU165512	*C*M haemovis	*Ovis aries*	1441
CanMyc56	EU165513	*C*M haemovis	*Ovis aries*	1441
CanMy135	EU828579	*C*M haemovis	*Ovis aries*	1345
CanMy133	EU828580	*C*M haemovis	*Ovis aries*	1345
CanMy134	EU828581	*C*M haemovis	*Ovis aries*	1345
MycSpe87	GU230141	*C*M haemovis	*Homo sapiens*	1439
MycSpe88	GU383116	*C*M haemovis	*Homo sapiens*	1439
MycSpe78	GU905011	*C*M haemozalophi	Sea lion	1438
MycSpe79	GU905012	*C*M haemozalophi	Sea lion	1438
CanMyco2	AF338269	*C*M kahanei	*Saimiri sciureus*	1451
CanMyc21	DQ157150	*C*M turicensis	Cat	1339
CanMyc22	DQ157151	*C*M turicensis	Cat	1336
CanMyc23	DQ157152	*C*M turicensis	Cat	1342
CanMyc24	DQ157153	*C*M turicensis	Cat	1442
CanMyc25	DQ157154	*C*M turicensis	Cat	1342
CanMyc26	DQ464417	*C*M turicensis	Cat	1343
CanMyc27	DQ464418	*C*M turicensis	Cat	1341
CanMyc28	DQ464419	*C*M turicensis	Cat	1340
CanMyc29	DQ464420	*C*M turicensis	Cat	1341
CanMyc30	DQ464421	*C*M turicensis	Cat	1341
CanMyc31	DQ464422	*C*M turicensis	Cat	1341
CanMyc32	DQ464423	*C*M turicensis	Cat	1394
CanMyc33	DQ464424	*C*M turicensis	Cat	1294
CanMyc34	DQ464425	*C*M turicensis	Cat	1294
CanMyc42	DQ825448	*C*M turicensis	*Leopardus pardali*	1306
CanMyc43	DQ825449	*C*M turicensis	European wildcat	1341
CanMyc44	DQ825450	*C*M turicensis	European wildcat	1341
CanMyc46	DQ825454	*C*M turicensis	Lion	1343
CanMyc60	EU532066	*C*M turicensis	Cat	1179
CanMyc61	EU789558	*C*M turicensis	Cat	1238
CanMyc62	EU789559	*C*M turicensis	Cat	1232
CanMyc63	EU839977	*C*M turicensis	*Felis catus*	1292
CanMyc71	EU861063	*C*M turicensis	*Felis catus*	1259
MycSpe32	AY831867	*C*M turicensis	Cat	1395
MycCavip	NR_024988	*Mycoplasma cavipharyngis*	Guinea pig	1444
MycCocco	AY171918	*Mycoplasma coccoides*	Mouse	1430
MycFast3	NR_024987	*Mycoplasma fastidiosum*	Horse	1455
HmbCanis	AF197337	*Mycoplasma haemocanis*	Dog	1397
HmcCani2	AF407208	*Mycoplasma haemocanis*	Dog	1393
MycHaem4	AY150973	*Mycoplasma haemocanis*	Dog	1430
MycHae10	AY529641	*Mycoplasma haemocanis*	Dog	1393
MycHae23	EF416566	*Mycoplasma haemocanis*	Dog	1330
MycHae24	EF416567	*Mycoplasma haemocanis*	Dog	1328

Table VIII-4 continued

ARB	Accession	Description	Host	bp
MycHae25	EF416568	*Mycoplasma haemocanis*	Dog	1330
MycHae33	GQ129115	*Mycoplasma haemocanis*	Dog	1283
MycHae34	GQ129116	*Mycoplasma haemocanis*	Dog	1283
MycHae35	GQ129117	*Mycoplasma haemocanis*	Dog	1283
MycHae36	GQ129118	*Mycoplasma haemocanis*	Dog	1283
MycHae37	EF416568	*Mycoplasma haemocanis*	Dog	1330
HmbFelis	AF178677	*Mycoplasma haemofelis*	Cat	1429
HmbFeli2	AY069948	*Mycoplasma haemofelis*	Cat	1393
HmbFeli3	U95297	*Mycoplasma haemofelis*	Cat	1377
MycHaem2	AF548631	*Mycoplasma haemofelis*	Cat	1396
MycHaem3	AY150972	*Mycoplasma haemofelis*	Cat	1396
MycHaem5	AY150976	*Mycoplasma haemofelis*	Cat	1396
MycHaem6	AY150977	*Mycoplasma haemofelis*	Cat	1396
MycHaem7	AY150984	*Mycoplasma haemofelis*	Cat	1396
MycHaem8	AY150985	*Mycoplasma haemofelis*	Cat	1396
MycHaem9	AY529632	*Mycoplasma haemofelis*	Cat	1312
MycHae11	DQ157155	*Mycoplasma haemofelis*	Cat	1328
MycHae12	DQ157156	*Mycoplasma haemofelis*	Cat	1330
MycHae13	DQ157157	*Mycoplasma haemofelis*	Cat	1322
MycHae14	DQ157158	*Mycoplasma haemofelis*	Cat	1330
MycHae15	DQ157159	*Mycoplasma haemofelis*	Cat	1330
MycHae16	DQ157160	*Mycoplasma haemofelis*	Cat	1330
MycHae17	DQ825438	*Mycoplasma haemofelis*	*Leopardus wiedii*	1330
MycHae18	DQ825441	*Mycoplasma haemofelis*	European wildcat	1329
MycHae19	DQ825447	*Mycoplasma haemofelis*	Iberian lynx	1328
MycHae20	DQ825451	*Mycoplasma haemofelis*	Lion	1333
MycHae21	DQ825453	*Mycoplasma haemofelis*	Lion	1330
MycHae22	DQ825458	*Mycoplasma haemofelis*	Eurasian lynx	1331
MycHae26	EU145745	*Mycoplasma haemofelis*	Cat	1452
MycHae27	EU442639	*Mycoplasma haemofelis*	*Felis catus*	1207
MycHae28	EU839978	*Mycoplasma haemofelis*	*Felis catus*	1283
MycHae29	EU930823	*Mycoplasma haemofelis*	*Felis catus*	1323
MycHae30	U88563	*Mycoplasma haemofelis*	Cat	1402
MycHae90	NC_014970	*Mycoplasma haemofelis*	Cat	1429
HmbMuris	U82963	*Mycoplasma haemomuris*	Wild mouse	1401
MycInso2	EU859974	*Mycoplasma insons*		1546
MycoOvis	AF338268	*Mycoplasma ovis*	Sheep, goat	1458
MycoOvi2	EU165509	*Mycoplasma ovis*	*Ovis aries*	1458
MycoOvi3	EU165510	*Mycoplasma ovis*	*Ovis aries*	1458
MycoOvi4	EU165511	*Mycoplasma ovis*	*Ovis aries*	1458
MycoOvi5	EU916726	*Mycoplasma ovis*	Sheep	1080
MycoOvi6	FJ440328	*Mycoplasma ovis*	Sheep	1079
MycoOv12	GU230143	*Mycoplasma ovis*	*Homo sapiens*	1457
MycoOv13	GU230142	*Mycoplasma ovis*	*Homo sapiens*	1457
MycOv14	GU230144	*Mycoplasma ovis*	*Homo sapiens*	1457
MycoOv15	EU828582	*Mycoplasma ovis*	*Ovis aries*	1362

Table VIII-4 continued

ARB	Accession	Description	Host	bp
MycoOv18	AB571119	*Mycoplasma ovis*	*Capricornis crispus*	993
MycPneu5	NC_000912	*Mycoplasma pneumoniae* M129		1513
MycSpe39	EF424082	*Mycoplasma* sp. China-1	Buffalo	1434
MycSpe90	GU124600	*Mycoplasma* sp. CSL 7666	Sea lion	1438
MycSpe91	GU124601	*Mycoplasma* sp. CSL 7755	Sea lion	1438
MycSpe92	GU124602	*Mycoplasma* sp. CSL 7750	Sea lion	1438
MycSpe93	GU124603	*Mycoplasma* sp. CSL 7783	Sea lion	1438
MycSpe94	GU124604	*Mycoplasma* sp. CSL 7873	Sea lion	1438
MycSpe95	GU124605	*Mycoplasma* sp. CSL 7881	Sea lion	1438
MycSpe96	GU124606	*Mycoplasma* sp. CSL 7758	Sea lion	1438
MycSpe97	GU124607	*Mycoplasma* sp. CSL C797	Sea lion	1438
MycSpe98	GU124608	*Mycoplasma* sp. CSL C606	Sea lion	1438
MycSpe99	GU124609	*Mycoplasma* sp. CSL 7897	Sea lion	1438
MycSp100	GU124610	*Mycoplasma* sp. CSL 7801	Sea lion	1438
MycSp101	GU124611	*Mycoplasma* sp. CSL 7822	Sea lion	1438
MycSp102	GU124612	*Mycoplasma* sp. CSL C795	Sea lion	1438
MycSp103	GU124613	*Mycoplasma* sp. CSL 7860	Sea lion	1438
MycSp104	GU124614	*Mycoplasma* sp. CSL 7871	Sea lion	1438
MycSp110	**FR668085**	***Mycoplasma* sp. horse 111**	**Horse**	**107**
MycSp111	**FR668086**	***Mycoplasma* sp. horse 127**	**Horse**	**106**
MycSp112	**FR668084**	***Mycoplasma* sp. horse 90**	**Horse**	**110**
MycSpe16	**FN421444**	***Mycoplasma* sp. Horse isolate 30/14**	**Horse**	**916**
MycSpe18	**FN421445**	***Mycoplasma* sp. Horse isolate 30/7**	**Horse**	**880**
MycSpe17	**FN421443**	***Mycoplasma* sp. Horse isolate 32/3**	**Horse**	**896**
MycSpe66	**FN392887**	***Mycoplasma* sp. Isolate BovHM-2**	**Cattle**	**978**
MycSpe67	**FN392888**	***Mycoplasma* sp. Isolate BovHM-5**	**Cattle**	**978**
MycSpe68	**FN392889**	***Mycoplasma* sp. Isolate BovHM-7**	**Cattle**	**978**
EperSuis	AF029394	*Mycoplasma suis* 'Zachary'	*Sus scrofa*	1374
EperSui2	U88565	*Mycoplasma suis* 'Illinois'	*Sus scrofa*	1436
MycoSuis	AY492086	*Mycoplasma suis* 'Guangdong'	*Sus scrofa*	1468
MycoSui2	EU603330	*Mycoplasma suis*	*Sus scrofa*	1473
MycoSui3	FJ263943	*Mycoplasma suis*	*Sus scrofa*	1473
MycoSui4	FJ263944	*Mycoplasma suis*	*Sus scrofa*	1473
MycoSui6	FN391022	*Mycoplasma suis*	*Sus scrofa*	1479
MycoSui7	FN391021	*Mycoplasma suis*	*Sus scrofa*	1479
MycoSui9	**FN391018**	***Mycoplasma suis***	**Wild boar**	**1009**
MycoSu10	**FN391020**	***Mycoplasma suis***	**Wild boar**	**1007**
MycoSu11	**FN391019**	***Mycoplasma suis***	**Wild boar**	**1008**
MyocSu12	**FN436016**	***Mycoplasma suis***	**Wild boar**	**1008**
MycoSu13	**FN436017**	***Mycoplasma suis***	**Wild boar**	**1008**
MycoSu14	**FN436018**	***Mycoplasma suis***	**Wild boar**	**1006**
MycoSu15	**FN436019**	***Mycoplasma suis***	**Wild boar**	**1002**
MycoSu16	**FN436009**	***Mycoplasma suis***	**Wild boar**	**972**
MycoSu17	**FN436010**	***Mycoplasma suis***	**Wild boar**	**1009**
MycoSu18	**FN436011**	***Mycoplasma suis***	**Wild boar**	**1006**
MycoSu19	**FN436012**	***Mycoplasma suis***	**Wild boar**	**1006**

Table VIII-4 continued

ARB	Accession	Description	Host	bp
MycoSu20	**FN436013**	***Mycoplasma suis***	**Wild boar**	**1005**
MycoSu21	**FN436014**	***Mycoplasma suis***	**Wild boar**	**1007**
MycoSu22	**FN436015**	***Mycoplasma suis***	**Wild boar**	**1003**
MycoSu34	HQ259257	*Mycoplasma suis* 'Zhejiang'	*Sus scrofa*	1473
MycoSu35	NC_015153	*Mycoplasma suis*	*Sus scrofa*	1469
EpeWenyo	AF016546	*Mycoplasma wenyonii*	Cattle	1394
MycWenyo	AY769937	*Mycoplasma wenyonii*	Cattle	1399
MycWeny2	AY946266	*Mycoplasma wenyonii*	Cattle	1471
MycWeny3	DQ641256	*Mycoplasma wenyonii*	Cattle	1334
MycWeny4	EF221880	*Mycoplasma wenyonii*	Cattle	1453
MycWeny5	EU367963	*Mycoplasma wenyonii*	Cattle	1374
MycWeny6	EU367964	*Mycoplasma wenyonii*	Cattle	1249
MycWeny7	FJ375309	*Mycoplasma wenyonii*	Cattle	1454
MycWeny8	**FN392885**	***Mycoplasma wenyonii***	**Cattle**	**1083**
MycWeny9	**FN392886**	***Mycoplasma wenyonii***	**Cattle**	**1004**
MycWen20	HM538187	*Mycoplasma wenyonii*	Cattle	1470
MycWen21	HM538188	*Mycoplasma wenyonii*	Cattle	1469
MycWen22	HM538189	*Mycoplasma wenyonii*	Cattle	1470
MycWen23	HM538190	*Mycoplasma wenyonii*	Cattle	1470
MycWen24	HM538191	*Mycoplasma wenyonii*	Cattle	1471
UncMyc20	AY837724	Uncultured *Mycoplasma* sp.	Mosquito midgut	1457
UncMyc48	EU888930	Uncultured *Mycoplasma* sp.	*Homo sapiens*	1214
UncMyc52	FJ667774	Uncultured *Mycoplasma* sp.	Capybara	1347
UncMyc53	FJ824847	Uncultured Mycoplasma sp.	Deer	1370
UncMyc54	FJ667773	Uncultured *Mycoplasma* sp.	Capybara	1347
UncMyc55	HQ183731	Uncultured *Mycoplasma* sp.	*Leopoldamys edwardsi*	1342
UncMyc56	HQ183732	Uncultured *Mycoplasma* sp.	*Leopoldamys edwardsi*	1360
UncMyc57	HQ197742	Uncultured *Mycoplasma* sp.	*Mazama nana*	1242
UncMyc58	HQ197743	Uncultured *Mycoplasma* sp.	*Blastoceros dichotomus*	1239
UncMyc59	HQ197744	Uncultured *Mycoplasma* sp.	*Mazama nana*	1246
UncMyc60	HQ197745	Uncultured *Mycoplasma* sp.	*Mazama nana*	1242
UncMyc61	HQ197746	Uncultured *Mycoplasma* sp.	*Blastoceros dichotomus*	1248
UncMyc62	HQ197747	Uncultured *Mycoplasma* sp.	*Blastoceros dichotomus*	1252
UncMyc63	HQ197748	Uncultured *Mycoplasma* sp.	*Mazama americana*	1253
UncMyc64	HQ197749	Uncultured *Mycoplasma* sp.	*Mazama nana*	1242

*C*M = *Candidatus* Mycoplasma; isolates sequenced during this study are highlighted in boldface

Alignment 16S rRNA of haemotrophic mycoplasmas

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                1          11         21         31         41         51         61         71         81         91       100
                |          |          |          |          |          |          |          |          |          |        |
CanMyc55      1 .......... AGAGUUUGAU -CCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGAA CGAGUAG--A ACUU-GUU-- CUGCUAGUGG     84
MycoOv13      1 .......... AGAGTTTGAT -CCTGGCTCA GGATTAATGC TGGTGGTATG CATAACACAT GCAAGTCGAA CGAGTAG--A ACTT-GTT-- CTGCTAGTGG     84
UncMyc63      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
CanMyl56      1 .......... AGAGTTTGAT -CCTGGCTCA GGATTAATGC TGGTGGTATG CATAACACAT GCAAGTCGAA CGAGTAG--A ACTT-GTT-- CTGCTAGTGG     84
CanMyl55      1 .......... AGAGTTTGAT -CCTGGCTCA GGATTAATGC TGGTGGTATG CATAACACAT GCAAGTCGAA CGAGTAG--A ACTT-GTT-- CTGCTAGTGG     84
MycWeny2      1 ACGCGUCGAC AGAGUUUGAU -CCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGAA CGAGUAG--A ACUU-GUU-- CUGCUAGUGG     94
UncMyc62      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
MycoSu35      1 .......... AGAGUUUGAU -UCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGAA CGAAAAAGGC CCUC-GGGUC UUUUUAGUGG     88
MycEryth      1 .......... AGAGUUUGAU -CCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGAA CGAUUUU-UA AGCA-AUUA- AAGAUAGUGG     86
CanMyco2      1 .........U AGAGUUUGAU -CCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGAA CGAAGAA--G U-UU--AC-- UUCUUAGUGG     83
MycSpe79      1 .......... .......... ........CA GGATTAACGC TGGTGGTATG CATAACACAT GCAAGTCGAA CGAAGGG--A GTTT-ACT-- CCCTTAGTGG     67
MycHaemo      1 .......... .......... ......CUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUCGUA CGAAGCU--A GCUU-GCU-- AGCUUAGUGG     69
CanMyco9      1 ---------- AGAGUUUGAU -CCUGGCUCA CGAUUAAUGC UGGCGGUAUG CAUAACACAU GCAAGUCGAA CGAAGAG--G GUUU-ACU-- CUCUUAGUGG     84
CanMyc10      1 .......... AGAGUUUGAU -CCUGGCUCA GGAUUAAUGC UGGUGGUAUG CAUAACACAU GCAAGUUGAA CGAAGAG--G GCUU-GCC-- CUCUUAGUGG     84
MycHae90      1 .......... .AGAGUUUGA UCCUAGCUCA GAAUUAAUGC UGAUGGUAUG CCUAAUACAU GCAAGUCGAA CGGAUCUUGG UUUC-GGCCA AGAUUAGUGG     88
HmbCanis      1 .......... .......... ......CUCA GAAUUAAUGC UGAUGGUAUG CCUAAUACAU GCAAGUCGAA CGGACCUUGG UUUC-GGCCA AGGUUAGUGG     73
CanMyc51      1 .......... AGAGUUUGAU -CCUGGCUCA GAAUUAAUGC UGAUGGUAUG CCUAAUACAU GCAAGUCGAA CGGACUUUGG UUUC-GGCCA AAGUUAGUGG     88
MycSpe32      1 .......... .......... ........CA GAAUUAACGC UGGUGGCAUG CCUAAUACAU GCAAGUCGAG CGAACUG--U CCAAAAGG-- CAGUUAGCGG     68
UncMyc52      1 .......... .......... .......... .......... .......... ........AT GCAAGTCGAA CGGACTT-TT CCTT-GGAA- AAGTTAGTGG     39
MycCocco      1 .......... AGAGUUUGAU -CCUGGCUCA GAAUUAACGC UGGUGGCAUG CCUAAUACAU GCAAGUCGAA CGAAUGUGCC CGCA-AGGGU ACGUUAGUGG     88
HmbMuris      1 .......... .......... ......CUCA GAAUUAACGC UGAUGGCAUA CCUAAUACAU GCAAGUCGAG CGGACCU-CU AGCA-AUAG- AGGUUAGCGG     71
MycCavip      1 .......... .......... .......... .........C TGGCGGCATG CCTAATACAT GCAAGTTGAA CGAAAGT--- AGCA-AT--- ACTTTAGTAG     54
MycFast3      1 .......... .......... .......... .........C TGGCGGCATG CCTAATACAT GCAAGTTGAA CGAGAGT--- AGCA-AT--- ATTCTAGTAG     54
MycInso2      1 .......... .......... .......... ...TTAACGC TGGCGGCATG CCTAATACAT GCAAGTTGAA CGAGAGT--- AGCA-AT--- ATTCTAGTAG     60
MycPneu5      1 ..UUUUUCUG AGAGUUUGAU -CCUGGCUCA GGAUUAACGC UGGCGGCAUG CCUAAUACAU GCAAGUCGAU CGAAAGU--- AGUA-AU--- ACUUUAGAGG     90

                101        111        121        131        141        151        161        171        181        191       200
                |          |          |          |          |          |          |          |          |          |        |
CanMyc55     85 CAAACGGGCG AGUAAUGCAU AUUUAACUUA CUUCUGCGAG UAGGAUAGCA GCCCGAAAGG GCUAUUAAUA CUACAUAG-G UUUAUGG--- -----AC---   172
MycoOv13     85 CAAACGGGCG AGTAATACAT ATTTAACTTA CTTCCGCGAG TAGGATAGCA GCCCGAAAGG GCTATTAATA CTACATAG-G TTTATGG--- -----AC---   172
UncMyc63      1 .....GGGCG AGT-ATACAT ATTTAACTTA CTTCCGCGAG TAGGATAGCA GCCCGAAAGG GCTATTAATA CTACATAG-G TTTATGG--- ------C---    81
CanMyl56     85 CAAACGGGCG AGTAATACAT ATTTAACTTA CTTCCGCGAG TAGGATAGCA GCCCGAAAGG GCTATTAATA CTACATAG-G TTTATGG--- -----AC---   172
CanMyl55     85 CAAACGGGCG AGTAATACAT ATTTAACTTA CCTTTACGAG GAGGATAGCA GTTCGAAAGG ACTATTAATA CTCCATAG-G TTTATAG--- -----AC---   172
MycWeny2     95 CAAACGGGCG AGUAAUACAU AUUUAACUUA CUUUUACGAG GAGGAUAGCA GUUCGAAAGG ACUAUUAAUA CUCCAUAG-G UUUA------ --UAAAC---   182
UncMyc62      1 .....GGGCG AGT-ATACAT ATTTAACTTA CTTTCGCGAA GAGAATAGCA GCCCGAAAGG GCTATTAATA CTCTATAG-G TTTATAG--- ----AA----    82
MycoSu35     89 CAAACGGGCG AGUAACGCAU ACUUAACUUA CUUAUCUGAG GAAAAUAGCA GCUCGAAAGA GCUAUUAAUA AUCCAUAG-G UUUAGGC-UA GAGG-AA-CU   184
MycEryth     87 CGAACGGGCG AGUAAUACAU AUCUAACUUA CUUAUGUGAG GGGAAUAGCA GCCCGAAAGG GCUAUUAAUG CUCCAUAG-G UUUA----U- ---A-GA---   173
CanMyco2     84 CAAACGGGCG AGUAAAACAU AUUCAACCUA CCUAUGCGAG GAGAAUAGCA ACUCGAAAGA GUUAUUAAUG GUCCAUAG-G UUUU----U- ---A-GA---   170
MycSpe79     68 CGAACGGGCG AGTAATACAT ATTTAACTTA CTCACGCGAG GAAGATAACT ACTCGAAAGA GTGGCTAATA ATCCATAG-G TTTGCAG-G- CTGCAAATTA   164
MycHaemo     70 CAAACGGGCG AGUAAUACAU AUUUAACUUA CUUACGCAAG GAAGAUAGCA ACUCGAAAGA GUUAUUAAUA CUCCAUAG-G UUUAGAU-U- U--G-AAA-U   162
CanMyco9     85 CGAACGGGCG AGUAACACAU AUUUAACUUA CCUGCGUGAG GAGAAUAGCA ACUCGAAAGA GUUAUUAAUA CUCCAUAG-G UUUA------ AU---UC-GU   173
CanMyc10     85 CGAACGGGCG AGUAACGCAU AUUUAACUUA CUUACGCGAG GAGAAUAGCA AUCCGAAAGG GUUAUUAAUA UUUCAUAG-G UUUA------ AG---A--CU   172
MycHae90     89 CAAACGGGUG AGUAAUACAU AUCUAACAUG CCCCUCUGUG GGGGAUAGCC GCUUGAAAAA GCGAUUAAUA CCCCAUAG-G AAGCUUU-A- UC---UAUGA   182
HmbCanis     74 CAAACGGGUG AGUAAUACAU AUCUAACAUG CCCCUCUGUG GGGGAUAGCC ACUUGAAAAA GUGAUUAAUA CCCCAUAG-G AAGCUUU-A- UC---CAUGA   167
CanMyc51     89 CGAACGGGUG AGUAAUACAU AUCUAACAUG CCCCUCUGUA GGGAAUAGCC ACUUGAAAAG GUAAUUAAUA CCCUAUAG-G UAACUUU-C- UC-A-CAAGA   183
MycSpe32     69 CGAACGGGUG AGUAAUACAU AUUUAACAUG CCCUCCGGAA GGAAAUAGCC GUUCGAAAGA ACGAUUAAUG UCCUAUAG-U AUCCUCC-A- UCAG-ACAGA   164
UncMyc52     40 CGAACGGGTG AGTAATGCAT ATTTAACATA CCCCTAGGAG GGGCATAGCC GCCTGAAAAG GCGATTAATA CCCCATAG-T AGCTCCC-T- CG---C-ATG   132
MycCocco     89 CGAACGGGUG AGUAAUACAU AUUUAACAUA CCCCUUAGAG GGAAAUAGCC GUCUGAAAAG ACGAUUAAUG UCCCAUAG-G AACCCCC-U- CA---C-AGG   181
HmbMuris     72 CGAACGGGUG AGUAAUGAAU ACUUAACAUA CCUCCAUGAA GGAAAUAGCU AUUCGAAAGA GUAAUUAAUG UCCUAUAG-G AGCCUUCCU- CA---C-AUG   165
MycCavip     55 CGAACGGGTG AGTAACACGT ATCCAATCTA CCCTTATGTA AGGAATAACC AGTTGAAAAA CTGGCTAATA CCTTATAG-G AGCATTA-A- AC-A-CAAG-   148
MycFast3     55 CGAACGGGTG AGTAACACGT ATCCAACCTA CCCTTATGTA AAGAATAACT AGATGAAAAT CTAGCTAATA CTTTATAG-G AGCATTT-A- AC-A-TAAGT   149
MycInso2     61 CGAACGGGTG AGTAACACGT ATCCAACCTA CCTTCATGTA AGGAATAACT AGTTGAAAAA TTAGCTAATA CCTTATAG-C AACATTA-A- AC-A-TAAGT   155
MycPneu5     91 CGAACGGGUG AGUAACACGU AUCCAAUCUA CCUUAUAAUG GGGGAUAACU AGUUGAAAGA CUAGCUAAUA CCGCAUAAGA ACUUUGG-U- UC-G-CAUGA   186

                201        211        221        231        241        251        261        271        281        291       300
                |          |          |          |          |          |          |          |          |          |        |
CanMyc55    173 -UU-GUAAAU UAAAG---GA UGCG--CCC- -UC--GGGAG CCUCGCGCGG AAAAGGAAAU AUAUCCUAUU AGGUAGUUGG CGGGGUAAAG GCCCACCAAG   261
MycoOv13    173 -TT-GTAAAT TAAAG---GA GGCG--CCC- -TC--GGGAG CCTCGCGCGG AAAAGGGAAT ATGTCCTATT AGGTAGTTGG CGGGGTAAAG GCCCACCAAG   261
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Alignment 16S rRNA of haemotrophic mycoplasmas

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UncMyc63    82  -TT-ATAAAT TAAAG---GA GGCG--CCT- -TC--GGGAG CCTTGCGCGG GAACGGGAAT ATGTCCTATT AGGTAGTTGG CGGGGTAAAG GCCCACCAAG   170
CanMy156   173  -TT-GTAAAT TAAAG---GA GGCG--CCC- -TC--GGGAG CCTCGCGCGG AAACGGGAAT ATGTCCTATT AGGTAGTTGG CGGGGTAAAG GCCCACCAAG   261
CanMy155   173  -TT-ATAAAT TAAAG---GG AGCT--CCG- -CA--AGGGG TTCCGCGTAA AAATAGGAAT GTGTCCTATT AGGTTGTTGG TGGGGTAAAG GCCTACCAAG   261
MycWeny2   183  -----UAAAU CAAA----GA GGCU--CCC- -UC--GGGGG CCUCGCGUGA AAAUAGGAAU AUGUCCUAUU AGGUAGUUGG CGGGGUAAAG GCCCACCAAG   267
UncMyc62    83  -AT-ATAAAT TAAAG---GA GGCG--CCT- -TC--GGGAG CCTTGCGCGA AAATAGGAAT ATGTCCTATT AGGTAGTTGG CGGGGTAAAG GCCCACCAAG   171
MycoSu35   185  AGC-UUAAAU UAAAG---GA GGCU--GCCG -CA-AGGUGG CCUUGCGGGU AAAUAGGAGU AUGUCCUAUU AGAUAGUUGG AGAGGUAAGG GCUCACCAAG   276
MycEryth   174  A----UAAAU UAAAU---GA GGCU--C-CG -CA-AG-GGG CCUCGCGCAU AAACAGGGAU AUGUCCUAUU AGGUAGUUGG UGGGGUAAAA GCCUACCAAG   260
CanMyco2   171  G----UAAAU UAAAG---GG CGCU--CCCG -AA-AGGGGG CGUUGCGCAU AGAUGGGAGU AUGUCCUAUU AGAUAGUUGG UGGGGUAAAG GCCUACCAAG   259
MycSpe79   165  AAT-GCGGAG TCCTTTCGAA GGCT--T--- -CGA---GAG CCGCGTGCGT GAATAGGAAT ATGTCCTATT AGGTAGTTGG TGGGATAAGA GCTTACCAAG   254
MycHaemo   163  AGU-CUAAAU UAAA----GG GGCG--CCG- -CA--AGGAG CCCUGUGCAU AAAUAGGAAU AUGUCCUAUU AGGUAGUUGG CGGGGUAAAG GCCCACCAAG   251
CanMyco9   174  -----UAAAU UAAAG---GG GGCGUUCCGG GUGACCGGAG CCUCGCGAGC AGAGAGGAGU AUGUCCUAUU AGGUUGUUGG UGGGGUAAAU GCUUACCAAG   265
CanMyc10   173  -----UAAAU UAAAG---GG GGCGUUCCU- -UC--GGGAG CCCCGCGCGU AAAUAGGAAU AUGUCCUAUU AGGUUGUUGG UGGGGUAAAU GCCUACCAAG   260
MycHae90   183  UUU-AGCUUU UAAA----GC -CU------- -UC------G G-GCGCUGAG GGAUUGGGAU AUGCUCUAUU AGCUAGUUGG CGGGAUAAAA GCCCACCAAG   261
HmbCanis   168  UUU-AGCUUU UAAA----GC -CU------- -UC------G G-GCGCUGAG GGAUUGGGAU AUGCUCUAUU AGCUAGUUGG CGGGAUAAAA GCCCACCAAG   246
CanMyc51   184  GUU-AGUUAU UAAA----GC -U-------- UUA------- U-GCGCUGAG GGAUUGGGAU AUGCUCUAUU AGCUUGUUGG CGGGGUAAAA GCCCACCAAG   261
MycSpe32   165  AGG-GGGAUU UAAA----GG -UG------- -AA------A A-CCGCCGGA GGAUUGGAAU AUGUCCUAUU AGCUAGUUGG CGGGAUAAAA GCCCACCAAG   243
UncMyc52   133  AGG-TAGCTT TAAA----GG -TT------- -TA------C T-CCGCCTAG GGATTGGAAT ATGTTCTACT AGTTTGTTGG TGAGGTAAAG GCTCACCAAG   211
MycCocco   182  AGGGGUUUUU UAAA----GG -AG------- -CA------A U-CCGCUUUG GGAUUGGAAU AUGCUCUAUU AGUUAGUUGG CGGGGUAAAG GCCCACCAAG   261
HmbMuris   166  AGGUUGGCUU UAAA----GG -CG------- -CA------A G-CCACUUGG AGAUUGGAGU AUUUUCUAUU AGCUAGUUGG CGGGAUAAUA GCCCACCAAG   245
MycCavip   149  TTT-TTGTTT TAAA----GA CGC---G--- -TT----T-G CGTTGCTTAA GGATGAGGGT GCGGTGCATT AGATAGTTGG CAGGGTAATG GCCTACCAAG   231
MycFast3   150  TTT-TTGTTT TAAA----GG CGC---G--- -TT----T-G CGTCGCTTAA GGATGGGGGT GCGGTGCATT AGATAGTTGG CGGGGTAATG GCCCACCAAG   232
MycInso2   156  TTT-TTGTTT TAAA----GA TCC---G--- -TT----A-G GATCGCTTGA AGATGGGGGT GCGGTGCATT AGATAGTTGG CGGGGTAATG GCCCACCAAG   238
MycPneu5   187  AUC-AAAGUU GAAA----GG ACCU--G--- -CA----AGG GUUCGUUAUU UGAUGAGGGU GCGCCAUAUC AGCUAGUUGG UGGGGUAACG GCCUACCAAG   271

                    301         311         321         331         341         351         361         371         381         391       400
                    |           |           |           |           |           |           |           |           |           |         |
CanMyc55   262  CCAAUGAUGG AUAGCUGGAC UGAGAGUUUG AACAGCCGCA AUGGGAUUGA GAUAUGGUCC AUAUUCCUAC GGGAAGCAAC AGUGAGGAAU UUUUCACAAU   361
MycoOv13   262  CCAATGATGG GTAGCTGGAC TGAGAGGTTG AACAGCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   361
UncMyc63   171  CCAATGATGG GTAGCTGGAC TGAGAGGTTG AACAGCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   270
CanMy156   262  CCAATGATGG GTAGCTGGAC TGAGAGGTTG AACAGCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   361
CanMy155   262  CCAATGATGG GTAGCTGAAC TGAGAGGTTG AACAGCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   361
MycWeny2   268  CCAGUGAUGG GUAGCUGGAC UGAGAGGUUG AACAGCCGCA AUGGGAUUGA GAUAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGAGGAAU UUUUCACAAU   367
UncMyc62   172  CCAGTGATGG GTAGATGGAC TGAGAGGTTG AACATCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   271
MycoSu35   277  UCGAUGAUGG GUAGCUGGAC UGAGAGGUUG AACAGCCGCA AUGGGAUUGA GAUAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGAGGAAU UUUUCACAAU   376
MycEryth   261  CCUGUGAUGG GUAGCUGGAC UGAGAGGUUG ACCAGCCGCA AUGGGAUUGA GAGAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGAGGAAU UUUUCACAAU   360
CanMyco2   260  UCGGCGAUGG GUAGCUGGGC UGAGAGGUCG UACAGCCGCA AUGGGAUUGA GAUAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGGGGAAU UUUUCACAAU   359
MycSpe79   255  CCGTTGATGG GTAGCTGGAC TGAGAGGTTG AACAGCCGCA ATGGGATTGA GATATGGCCC ATATTCCTAC GGGAAGCAGC AGTGAGGAAT TTTTCACAAT   354
MycHaemo   252  CCGGUGAUGG GUAGCUGAAC UGAGAGGUUG GACAGCCGCA AUGGGAUUGA GAUAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGAGGAAU UUUUCACAAU   351
CanMyco9   266  CCGAUGAUGG GUAGCUGGAC UGAGGGGUCG ACCAGCCGCA AUGGGAUUGA AAUACGGCCC AUAUUCCUAC GGGAUGCAGC AGUGAGGAAU UUUUCACAAU   365
CanMyc10   261  CCGUUGAUGG GUAGCUGGGC UGAGAGGCCG ACCAGCCGCA AUGGGAUUGA AAUAUGGCCC AUAUUCCUAC GGGAAGCAGC AGUGAGGAAU UUUUCACAAU   360
MycHae90   262  GCAAUGAUAG AUAGCUGGUC UUAGAGGAUG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   361
HmbCanis   247  GCAAUGAUAG AUAGCUGGUC UUAGAGGAUG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   346
CanMyc51   262  GCUAUGAUAG AUAGCUGGUC AUAGAGGAUG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   361
MycSpe32   244  GCGAUGAUAG GUAGCUGGUC UAAGAGGAUG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   343
UncMyc52   212  ACGATGATAG ATAGCTGGTC TTAGAGGATG AACAGCCACA ATGGGATTGA GATACGGCCC ATATTCTTAC GGGAAGCAGC AGTAGGGAAT CTTCCACAAT   311
MycCocco   262  ACUAUGAUAG AUAGCUGGUC UUAGAGGACG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   361
HmbMuris   246  GCAGUGAUAG AUAGCUGGUC UAAGAGGAUG AACAGCCACA AUGGGAUUGA GAUACGGCCC AUAUUCCUAC GGGAAGCAGC AGUAGGGAAU CUUCCACAAU   345
MycCavip   232  TCGATGATGC ATAGCTGTAC TGAGAGGTAG AACAGCCACA ATGGGACTGA GACACGGCCC ATATTCCTAC GGGAAGCAGC AGTAGGGAAT TTTCCACAAT   331
MycFast3   233  TCGATGATGC ATAGCTGTAC TGAGAGGTAG AACAGCCACA ATGGGACTGA GACACGGCCC ATATTCCTAC GGGAAGCAGC AGTAGGGAAT TTTCCACAAT   332
MycInso2   239  TCGATGATGC ATAGCTGTAC TGAGAGGTAG AACAGCCACA ATGGGACTGA GACACGGCCC ATATTCCTAC GGGAAGCAGC AGTAGGGAAT TTTCCACAAT   338
MycPneu5   272  GCAAUGACGU GUAGCUAUGC UGAGAAGUAG AAUAGCCACA AUGGGACUGA GACACGGCCC AUACUCCUAC GGGAGGCAGC AGUAGGGAAU UUUUCACAAU   371

                    401         411         421         431         441         451         461         471         481         491       500
                    |           |           |           |           |           |           |           |           |           |         |
CanMyc55   362  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAAUGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-- AGCG------ ----------   442
MycoOv13   362  GGACGAAAGT CTGATGGAGC AATACCACGT GAACGATGAA GGTCTT-CTG ATTGTAAAGT TCTTTTATTT AGGAAAAA-A AGCGCGC--- TAGGAAATGA   456
UncMyc63   271  GGACGAAAGT CTGATGGAGC AATACCACGT GAGCGATGAC GGTCTT-CTG ATTGTAAAGC TCTTTTATTT AGGAAAAA-A AGCGCGC--- TAGGAAATGA   365
CanMy156   362  GGACGAAAGT CTGATGGAGC AATACCACGT GAACGATGAA GGTCTT-CTG ATTGTAAAGT TCTTTTATTT AGGAAAAA-A AGCGCGC--- TAGGAAATGA   456
CanMy155   362  GGACGAAAGT CTGATGGAGC AATACCACGT GAACGATGAA GGTCTT-TTG ATTGTAAAGT TCTTTTATTT AGGAAAAA-A AGCGCGC--- TAGGAAATGA   456
MycWenv2   368  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAACGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-A AGCGCGC--- UAGGAAAUGA   462
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Alignment 16S rRNA of haemotrophic mycoplasmas

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UncMyc62   272  GGACGAAAGT CTGATGGAGC AATACCACGT GAACGATGAA GGCCTT-CTG GTTGTAAAGT TCTTTTATTT AGGAAAAA-A AGCGCGC--- CAGGAAATGG   366
MycoSu35   377  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAACGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-A AGCGCUA--- CAGGAAAUGG   471
MycEryth   361  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAACGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUACUU AGGAAAAA-A AGCUUGA--- CAGGAAAUGG   455
CanMyco2   360  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAGCGAUGAA UGUCUU-CUG AUUGUAAAGC UCUUUUAUUU AGGAUAAA-A AGCAUGA--- UAGGAAAUGA   454
MycSpe79   355  GGACGAAAGT CTGATGAAGC AATACCACGT GAATGATGAA GGCCTT-CTG GTTGTAAAGT TCTTTTATTT AGGAAAAA-A ATCACGA--- TAGGAAATGA   449
MycHaemo   352  GGGCGAAAGC CUGAUGGAGC AAUACCACGU GAACGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-A AGCAGGA--- UAGGAAAUGA   446
CanMyco9   366  GGACGAAAGU CUGAUGGAGC AAUACCAUGU GAACGAUGAA GGUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-A AGCUUGA--- GAGGAAAUGA   460
CanMyc10   361  GGACGAAAGU CUGAUGGAGC AAUACCACGU GAACGAUGAA GAUCUU-CUG AUUGUAAAGU UCUUUUAUUU AGGAAAAA-A AGCUUGA--- UAGGAAAUGA   455
MycHae90   362  GGACGAAAGU CUGAUGGAGC AAUACCAUGU GAACGAUGAA GGCCUUUUUG GUUGUAAAGU UCUUUUACGA GGGAUA---- ---------- ---AUUAUGA   444
HmbCanis   347  GGACGAAAGU CUGAUGGAGC AAUACCAUGU GAACGAUGAA GGCCUUUUUG GUUGUAAAGU UCUUUUACGA GGGAUA---- ---------- ---AUUAUGA   429
CanMyc51   362  GGACGAAAGU CUGAUGGAGC AAUACCAUGU GAACGAUGAA GGUCUUUUUG AUUGUAAAGU UCUUUUAUGA GGGAUA---- ---------- ---ACAACUG   444
MycSpe32   344  GGGCGAAAGC CUGAUGGAGC AAUGCCAUGU GAACGAUGAA GGCCAGACAG GUCGUAAAGU UCUUUUAGAG GGGAAA---- ---------- ---AAUUUGA   426
UncMyc52   312  GGACGAAAGT CTGATGGAGC AATGCCATGT GAACGATGAA GGCCTATTTG GTCGTAAAGT TCTTTTAGGA GGGAAG---- ---------- ---ACTTTGA   394
MycCocco   362  GGACGAAAGU CUGAUGGAGC AAUGCCAUGU GAACGAUGAA GGUCAUUUUG AUUGUAAAGU UCUUUUAGGA GGGAAA---- ---------- ---AUUAUGA   444
HmbMuris   346  GGGCGAAAGC CUGAUGGAGU GAUGCCAUGU GAACGAUGAA GGUCUUUUUG AUUGUAAAGU UCUUUUAUUG GGGAAA---- ---------- ---AUGAUGA   428
MycCavip   332  GGGCGAAAGC CTGATGGAGC AATGCCGCGT GAATGATGAC GACCCTATGG GTTGTAAAAT TCTTTTATTA GGAAAAAATG ATTAGAAGAG ---GA-AA--   425
MycFast3   333  GGGCGAAAGC CTGATGGAGC AATGCCGCGT GAGTGATGAC GGCCCTTTGG GTTGTAAAAC TCTTTTATTA GGAAAAAATG AACAGAAGAG ---GA-AA--   426
MycInso2   339  GGGCGAAAGC CTGATGGAGC AATGCCGCGT GAGTGATGAC RGCCCTCTGG GTTGTAAAGC TCTTTTATTA GGAAAAAATA AACATGCTAG ---GA-AA--   432
MycPneu5   372  GAGCGAAAGC UUGAUGGAGC AAUGCCGCGU GAACGAUGAA GGUCUUUAAG AUUGUAAAGU UCUUUUAUUU GGGAAGAAUG ACUUUAGCAG ---GU-AA--   465

                501         511         521         531         541         551         561         571         581         591       600
                |           |           |           |           |           |           |           |           |           |         |
CanMyc55   443  ------CGCC UUGAUGGUAC UA-AUUGAAU AAGUGACAGC UAACUAUGUG CCAGUAGUUG CGGUAAAACA UAGGUCACAA GCAUUAUCCG GAUUUAUUGG   535
MycoOv13   457  ---GCGCGCC TTGATGGTAC TA-ATTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   552
UncMyc63   366  ---GCGCGCC TTGATGGTAC TA-ATTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   461
CanMyl56   457  ---GCGCGCC TTGATGGTAC TA-ATTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   552
CanMyl55   457  ---GCGCGCC TTGATGGTAC TA-ATTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   552
MycWeny2   463  ---GCGCGCC UUGAUGGUAC UA-AUUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAAAACA UAGGUCACGA GCAUUAUCCG GAUUUAUUGG   558
UncMyc62   367  ---GTGCGCC TTGATTGTAC TA-TTTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   462
MycoSu35   472  ---UCGCGCC CUGAUUGUAC UA-AUUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAAAACA UAGGUCACGA GCAUUAUCCG GAUUUAUUGG   567
MycEryth   456  ---UUAAGCC UUGAUCGUAC UA-GGUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAAACAA UAGGUCACAA GCAUUAUCCG GAUUUAUUGG   551
CanMyco2   455  ---UUAUGCC UUGAUCGUAC UA-AAUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAACACA UAGGUCACAA GCAUUAUCCG GAUUUAUUGG   550
MycSpe79   450  ---TTGAGAT TTGATCGTAC TA-ATTGAAT AAGTGACAGC TAACTATGTG CCAGCAGCTG CGGTAAAACA TAGGTCACAA GCATTATCCG GATTTATTGG   545
MycHaemo   447  ---UUCUGUC GUGAUUGUAC UA-AUUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAAAACA UAGGUCACGA GCAUUAUCCG GAUUUAUUGG   542
CanMyco9   461  ---UUAAGCC UUGAUUGUAC UA-GAUGAAU AAGUGACAAC UAACUAUGUG CCAGCAGCUG CGGUAAAACA UAGGUCACGA GCAUUAUCCG GAUUUAUUGG   556
CanMyc10   456  ---UUAAGCC UUGAUCGUAC UA-AAUGAAU AAGUGACAGC UAACUAUGUG CCAGCAGCUG CGGUAAAACA UAGGUCACGA GCAUUAUCCG GAUUUAUUGG   551
MycHae90   445  ---------- ----UAGUAC UU-CGUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCGA GCAUUAUUCG GAUUUAUUGG   529
HmbCanis   430  ---------- ----UAGUAC UU-CGUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCAA GCAUUAUUCG GAUUUAUUGG   514
CanMyc51   445  ---------- ---AUAGUAC CU-CAUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCAA GCAUUAUUCG GAUUUAUUGG   530
MycSpe32   427  ---------- ----UGGUAC CC-UCUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCGA GCGUUAUUCG GAUUUAUUGG   511
UncMyc52   395  ---------- ----CGGTAC CT-CCTGAAT AAGTGACAGC AAACTATGTG CCAGCAGCTG CGGTAATACA TAGGTCGCGA GCGTTATTCG GTTTTATTGG   479
MycCocco   445  ---------- ----UGGUAC CU-CCUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCAA GCGUUAUUCG GAUUUAUUGG   529
HmbMuris   429  ---------- ----UGGUAC CC-AGUGAAU AAGUGACAGC AAACUAUGUG CCAGCAGCUG CGGUAAUACA UAGGUCGCGA GCGUUAUUCG GAUUUAUUGG   513
MycCavip   426  TGCTTCTAAT TTGATTGTAC CT-TTTGAAT AAGCAACGGC TAACTATGTG CCAGCAGCCG CGGTAATACA TAGGTTGCAA GCGTTATCCG GATTTATTGG   524
MycFast3   427  TGCTTCTGTT TTGATTGTAC CT-TTTGAAT AAGCAACGGC TAACTATGTG CCAGCAGCCG CGGTAATACA TAGGTTGCAA GCGTTATCCG GATTTATTGG   525
MycInso2   433  TGAGCATGTY TTGATTGTAC CT-TTTGAAT AAGCAACGGC TAACTATGTG CCAGCAGCCG CGGTAATACA TAGGTTGCAA GCGTTATCCG GATTTATTGG   531
MycPneu5   466  UGGCUAGAGU UUGACUGUAC CAUUUUGAAU AAGUGACGAC UAACUAUGUG CCAGCAGUCG CGGUAAUACA UAGGUCGCAA GCGUUAUCCG GAUUUAUUGG   565

                601         611         621         631         641         651         661         671         681         691       700
                |           |           |           |           |           |           |           |           |           |         |
CanMyc55   536  GCGUAAAGGA AGCGUAGGCG GGGAGGCUGA UCCAUUGUUA AAGGCAUUUG CUCAAC-AAA UGUGUGCGAU GGAGAUCUCC UCCUUAGAGU UAAUCAAGGG   634
MycoOv13   553  GCGTAAAGGA AGCGTAGGCG GGGAGGCTGA TCCATTGTTA AAGGCATTTG CTCAAC-AAA TGTGTGCGAT GGAGATCTCC TCCCTAGAGT TAATCAGGGG   651
UncMyc63   462  GCGTAAAGGA AGCGTAGGCG GGGAGGTTGA TCCATTGTTA AAGGCATTTG CTCAAC-AAA TGTGTGCGAT GGAAATCGCC TCCCTAGAGT TAATCAGGGG   560
CanMyl56   553  GCGTAAAGGA AGCGTAGGCG GGGAGGTTGA TCCATTGTTA AAGGCATTTG CTCAAC-AAA TGTGTGCGAT GGAGATCGCC TCCCTAGAGT TAATCAGGGG   651
CanMyl55   553  GCGTAAAGGA AGCGTAGGCG GGGAGGTCGA TCCATTGTTA AAGGCATTTG CTTAAC-AAA TGTATGCGAT GGAGATCGCC TTCCTAGAAT TAATCAGGGG   651
MycWeny2   559  GCGUAAAGGA AGCGUAGGUG GGGAGGUUGA UCCAUUGUUA AAGGCAUUUG CUUAAC-AAA UGUGUGCGAU GGAGAUCGCC UCCCUAGAGU UAAUCAGGGG   657
UncMyc62   463  GCGTAAAGGA AGCGTAGGCG GAGGAGATGA TCCATTGTTA AAGGCATTTG CTTAAC-AAA TGTTCGCGAT GGATATCACT TCTCTAGAAT TAATCAGGGG   561
MycoSu35   568  GCGUAAAGGA AGCGUAGGCU GAAGUGUGUA UCCAUUGUUA AAAGUACUUG CUUAAC-AAG UGUUCGCGGU GGAGAUUACA CUUCUAGAAU UAGUUAGAGG   666
MycEryth   552  GCGUAAAGGA AGCGUAGGCG GACAGUCUGA UCCAUUGUUA AAUACAUUUG CUCAAC-AAA UGUAUGCAGU GGAGAUCUCC UGUCUAGAAU UAAUUAGGGG   650
CanMyco2   551  GCGUAAAGAA AGCGUAGGCG GAGAGUUUGA UCUAGUGUUA AAUGCACCUG CUCAAC-AGG UGCAUGCAUU AGAAAUCAGC UAUCUAGAAU UAGUUAGAGG   649
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Alignment 16S rRNA of haemotrophic mycoplasmas

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MycSpe79   546  GCGTAAAGGA AGCGTAGGCG GAAGAGTTGA TCCAGTGTTA AAGGCATCTG CTTAAC-AGG TGTTTGCATT GGAGATCGCT CTTCTAGAAT TAGTTAGGGG   644
MycHaemo   543  GCGUAAAGGA AGCGUAGGUG GAGGAGUUGA UCCAUUGUCA AAAGCAUCUG CUUAAC-AGG UGUCCGCGAU GGAUAUCGCU UCUCUAGAAU UAGUUAGGGG   641
CanMyco9   557  GCGUAAAGGA AGCGUAGGCG GACAGGUUGA UCUAUUGUGA AAGGCACUUG CUCAACGAGU UGUUUGUGAU AGAUAUCGUU UGUCUAGAAU UAGUUAGGAG   656
CanMyc10   552  GCGUAAAGGA AGCGUAGGCG GACAAGUUGA UCUAUUGUGA AAAGCACUUG CUCAACAAGU UGUUUGUGAU AGAUAUCGCU UGUCUAGAAU UAGUUAGGAG   651
MycHae90   530  GCGUAAAGCA AGCGCAGGCG GAUGUGUAAG UUCUGUGUUA AAUGCAGCUA CUCAAU-AGU UGUAUGCACC GAAUACUACA UGUCUAGAUU GUGGUAGGGA   628
HmbCanis   515  GCGUAAAGCA AGCGCAGGCG GAUGUGUAAG UUCUGUGUUA AAUGCAGCUA CUCAAU-AGU UGUAUGCACC GAAUACUACA UGUCUAGAUU GUGGUAGGGA   613
CanMyc51   531  GCGUAAAGCA AGCGCAGGCG GAUGUGUAAG UUCUGUGUUA AAAGUAGCUA CUUAAU-AGU UGUUUGCACC GAAUACUACA UGUCUAGAAU GUGGUAGGAA   629
MycSpe32   512  GCGUAAAGCA AGCGCAGGCG GAUGAAUAAG UUCUGCAUUA AAAGCAGCUG CUUAAC-AGU UGUUUGUGCC GAAUACUAUU CAUCUAGAAU GUGGUAGGAA   610
UncMyc52   480  GCGTAAAGCA AGCGCAGGCG GATGAACAAG TTCTGTGTTA AAAGCAGCTG CTCAAC-AGT TGTTTGCACC GAATACTGTT CGTCTAGAAT GTGGTAGGAA   578
MycCocco   530  GCGUAAAGCA AGCGCAGGCG GAUGAACAAG UUCUGUGUUA AAAGCAGCUG CUCAAC-AGU UGUUUGCACC GAAUACUGUU CGUCUAGAAU GUGGUAGGAA   628
HmbMuris   514  GCGUAAAGCG AGCGCAGGCG GAUUGGUAAG UUCUGUGUUA AAUGCAGCCG CUCAAC-GGU UGUAUGCGCA GAAUACUGCC UUUCUAGAAU ACGGUAGAAA   612
MycCavip   525  GCGTAAAGCA AGCGCAGGCG GAGTAACAAG TCTGGTGTTA AAGGCAATAG CTTAAC-TAT TGTTTGCATT AGAAACTGTT AATCTAGAAT ACAGTAGGGA   623
MycFast3   526  GCGTAAAGCA AGCGCAGGTT GATTAACAAG TCTGGTGTGA AAGGCAGTAG CTTAAC-TAT TGTTTGCATT AGAAACTGTT AATCTAGAAT ACAGTAGGGA   624
MycInso2   532  GCGTAAAGCA AGCGCAGGTT GGTTATCAAG TCTAGTGTGA AAGGCAATTG CTTAAC-AAT TGTTTGCATT AGAAACTGCT AACCTAGAAT ACAGTAGGGA   630
MycPneu5   566  GCGUAAAGCA AGCGCAGGCG GAUUGAAAAG UCUGGUGUUA AAGGCAGCUG CUUAAC-AGU UGUAUGCAUU GGAAACUAUU AAUCUAGAGU GUGGUAGGGA   664

                701         711         721         731         741         751         761         771         781         791       800
                |           |           |           |           |           |           |           |           |           |         |
CanMyc55   635  GUACUGGAAU UCAAUGUGUA GUGGUGGAAU GCGUAGAUGU AUUGAGGAAA ACCAGAGGCU AAGGCGAGUA CCUGGGAUAU A-ACUGACGC UGAGGCUUGA   733
MycoOv13   652  GTACTGGAAT TCAATGTGTA GCGGTGGAAT GCGTAGATAT ATTGAGGAAA ACCAGAGGCT AAGGCGAGTA CCTGGGATAT A-ACTGACGC TGAGGCTTGA   750
UncMyc63   561  GTACTGGAAT TCAATGTGTA GCGGTGGAAT GCGTAGATAT ATTGAGGAAC ACCAGAGGCT AAGGCGAGTA CCTGGGATAT A-ACTGACGC TGAGGCTTGA   659
CanMy156   652  GTACTGGAAT TCAATGTGTA GCGGTGGAAT GCGTAGATAT ATTGAGGAAC ACCAGAGGCT AAGGCGAGTA CCTAGGATTA A-ACTGACGC TGAGGCTTGA   750
CanMy155   652  GCACTGGAAT TCAATGTGTA GCGGTGGAAT GCGTAGATAT ATTGAGGAAC ACCAGAGGCT AAGGCGAGTG CCTAGGATTT A-ATTGACGC TGAGGCTTGA   750
MycWeny2   658  GUACUGGAAU UCAAUGUGUA GCGGUGGAAU GCGUAGAUAU AUUGAGGAAC ACCAGAGGCU AAGGCGAGUA CCUAGGAUAU A-ACUGACAC UGAGGCUUGA   756
UncMyc62   562  GTACTGGAAT TCAATGTGGA GCGGTGGAAT GCGTAGATAT ATTGAGGAAC ACCAGAGGCT AAGGCGAGTA CCTGGGAGAT A-ATTGACGC TGAGGCTTGA   660
MycoSu35   667  GCACUGGAAU UCAAUGUGUA GUGGUGGAAU ACGUAGAUAU AUUGAGGAAC ACCGGAGGCU AAGGCGAGUG CCUGGGACAU A-AUUGACGC UGAGGCUUGA   765
MycEryth   651  AAACUGGAAU UCAAUGUGUA GUGGUGGAAU ACGUAGAUAU AUUGAGGAAC ACCAAAGGCG AAAGCAAGUU UCUAGAAUAU A-AUUGACGC UGAGGCUUGA   749
CanMyco2   650  GUACUGGAAU UCAAUGUGGA GUGGUGGAAU ACGUAGAUAU AUUGAGGAAC ACCAGAGGCU AAGGCGAGUA CCUGGGACAU A-AUUGACGC UAAGGCUUGA   748
MycSpe79   645  GTACTGGAAT TCAATGTGTA GCGGTGGAAT GCTTAGATAT ATTGAGGAAC ACCAGAGGCT AAGGCGAGTG CCTGGAACAT A-ATTGACGC TGAGGCTTGA   743
MycHaemo   642  GUACUGGAAU UCAAUGUGUA GCGGUGGAAU GCGUAGAUAU AUUGAGGAAC ACCAGAGGCU AAGGCGAGUA CCUGGGACAU A-AUUGACAC UGAGGCUUGA   740
CanMyco9   657  AUACUGGAAU UCAAUGUGUA GCGGUGGAAU GCGUACAUAU AUUGAGGAAC ACCAGAGGCU AAGGCGAGUA UCUAGGACAU A-AUUGACGC UGAGGCUUGA   755
CanMyc10   652  AUACUGGAAU UCAAUGUGUA GCGGUGGAAU GCGUAGAUAU AUUGAGGAAC ACCAGAGGCU AAGGCGAGUA UCUAGAACAU A-AUUGACGC UGAGGCUUGA   750
MycHae90   629  GUUUCGGAAU UAAGCAUGGA GCGGUGGAAU GUGUAGAUAU GCUUAAGAAC ACCAGAGGCG AAGGCGGAAA CUUAGGCC-A UAAAUGACGC UUAGGCUUGA   727
HmbCanis   614  GUUUCGGAAU UAAGCAUGGA GCGGUGGAAU GUGUAGAUAU GCUUAAGAAC ACCAGAGGCG AAGGCGGAAA CUUAGGCC-A UAAAUGACGC UUAGGCUUGA   712
CanMyc51   630  GUUUUGGAAU UAAACAUGGA GCGGUGGAAU GUGUAGAUAU GUUUAAGAAC ACCGGAGGCG AAGGCGAAAA CUUAGGCC-A UAAUUGACGC UUAGGCUUGA   728
MycSpe32   611  GUUUUGGAAU UAAAUAUGGA GCGGUGGAAU GUGUAGAUAU AUUUAAGAAC ACCAGAGGCG AAGGCGAAAA CUUAGGCCAU U-AUUGACGC UUAGGCUUGA   709
UncMyc52   579  GTTTTGGAAT TAAATATGGA GCGGTGGAAT GTGTAGATAT ATTTAAGAAC ACCAGAGGCG AAGGCGAAAA CTTAGGCC-A TCATTGACGC TTAGGCTTGA   677
MycCocco   629  GUUUUGGAAU UAAAUAUGGA GCGGUGGAAU GUGUAGAUAU AUUUAAGAAC ACCAGAGGCG AAGGCGAAAA CUUAGGCC-A UUAUUGACGC UUAGGCUUGA   727
HmbMuris   613  GUUUUGGAAU UGAAUGUGGA GCGGUGGAAU GUGUAGAUAU AUUCAAGAAC ACCAGAGGCG AAGGCGAAAA CUUAGGCC-G AUAUUGACGC UUAGGCUCGA   711
MycCavip   624  GTTCTGGAAT TCAATGTGGA GCGGTGAAAT GCGTAGATAT ATTGAAGAAC ACCAGTGGCG AAGGCGAGAA CTTGGGCT-G TAATTGACGC TTAGGCTTGA   722
MycFast3   625  GTTCTGGAAT TCAATGTGGA GCGGTGAAAT GCGTAGATAT ATTGAAGAAC ACCAGTGGCG AAGGCGAGAA CTTGGGCT-G TTATTGACGC TTAGGCTTGA   723
MycInso2   631  GTTCTGGAAT TCAATGTGGA GCGGTGGAAT GCGTAGATAT ATTGAAGAAC ACCAGTGGCG AAGGCGAGAA CTTGGGCT-G TTATTGACGC TTAGGCTTGA   729
MycPneu5   665  GUUUUGGAAU UUCAUGUGGA GCGGUGAAAU GCGUAGAUAU AUGAAGGAAC ACCAGUGGCG AAGGCGAAAA CUUAGGCC-A UUACUGACGC UUAGGCUUGA   763

                801         811         821         831         841         851         861         871         881         891       900
                |           |           |           |           |           |           |           |           |           |         |
CanMyc55   734  AAACGUGGGG AGUAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGAGC AUUAGGUAUU UGAU--GUUA G--GUCGAGU GCUGUAGCUA   827
MycoOv13   751  AAGCGTGGGG AGTAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGAGC ATTAGGTATT TGAT--GTTA G--GTCGAGT GCTGTAGCTA   844
UncMyc63   660  AAGCGTGGGG AGCAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGAGA ATTAGGTATT TGAT--GTTA T--ATCGAGT GCTGTAGCTA   753
CanMy156   751  AAGCGTGGGG ATCAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGAGC ATTAGGTATT TGAT--GTTA T--GTCGAGT GCTGTAGCTA   844
CanMy155   751  AAGCGTGGGG AGCAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGAGC ATTAGGTATT TGGC--ATTA G--GCCGAGT GCTGTAGCTA   844
MycWeny2   757  AAGCGUGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUG AACGAUGGGC AUUAGGUAUU UGAC--AUCU A--GUCGAGU GCUGUAGCUA   850
UncMyc62   661  AAGCGTGGGG AGCAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGGGT ATTAGGTATT TGAT--TTAA T--ATTGAGT GCTGTAGCTA   754
MycoSu35   766  AAGCGUGGGU AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGUCAUU UGAA--UUUA A--GUUGAGU GAUGUAGCUA   859
MycEryth   750  AAGCGUGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUUGGCAUU UGGG--CUGG A--CUUGAGU GCCGGAGCUA   843
CanMyco2   749  AAGCGUGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGGUAUU UGGA--UGAU G--UCUGAGU GCUGUAGCUA   842
MycSpe79   744  AAGCGTGGGG AGCAAATGGG -ATTAGATAC CCCAGTAG-T CCACGCCGTA AACGATGGGT ATTAAGTATT TGATTATCGT AAAATTGAGT GCTGTAGCTA   841
MycHaemo   741  AAGCGUGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGGUAUU UGGU--UUAA A--ACUGAGU GCUGUAGCUA   834
CanMyco9   756  AAGCGUGGGA AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGGUAUU UGGU--UAAG G--ACUGAGU GCUGUAGCUA   849
CanMyc10   751  AAGCGUGGGG AGCAAAUGGG AAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGGUAUU UGAU--AUAA G--AUUGAGU GCUGUAGCUA   845
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Alignment 16S rRNA of haemotrophic mycoplasmas

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MycHae90   728  AAGUGUGGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGC-UUUA --GCUUUAGU GUUGUAGCUU   822
HmbCanis   713  AAGUGUGGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGC-UUUA --GCUUUAGU GUUGUAGCUU   807
CanMyc51   729  AAGUGUGGGGG AGCAAGUGGG -AUUAGAUAC CCCAGUAGUU CCACACCGUA AACGAUGGGU AUUAGGUAUU GGGGU-UUGA --GCCUCAGU GCUGUAGCUA   824
MycSpe32   710  AAGUGUGGGGU AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUGUC GGGAU-UUGU --GUUUCGGC GUUGUAGCUU   804
UncMyc52   678  AAGTGTGGGT AGCAAATGGG -ATTAGATAC CCCAGTAG-T CCACACCGTA AACGATGGGT ATTGGATGTC GGGCT-TTGC --GGCTCGGT GTTGTAGCTT   772
MycCocco   728  AAGUGUGGGU AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUGCC GGGGU-UAGA --GCUUCGGU GCUGUAGCUU   822
HmbMuris   712  AAGUGUGGGG AGCAAAUGGG -AUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGAU AUUAGAUGUU GGGAC-UUGA --GUCUCAGC GUUGUAGCUU   806
MycCavip   723  AAGTGTGGGG AGCAAATAGG -ATTAGATAC CCTAGTAG-T CCACACTGTA AACGATGGAT ATTAGTTGTT GGGAC-TAGA --GTCTCAGT GACGCAGCTA   817
MycFast3   724  AAGTGTGGGG AGCAAATAGG -ATTAGATAC CCTAGTAG-T CCACACTGTA AACGATGGAT ATTAGTTGTT GGGAC-TAGA --GTCTCGGT GACGCAGCTA   818
MycInso2   730  AAGTGTGGGG AGCAAATAGG -ATTAGATAC CCTAGTAG-T CCACACTGTA AACGATGGAT ATTAGTTGTT GGGGC-TAGA --GCCTCGGT GACGTAGCTA   824
MycPneu5   764  AAGUGUGGGG AGCAAAUAGG -AUUAGAUAC CCUAGUAG-U CCACACCGUA AACGAUAGAU ACUAGCUGUC GGGGC-GAUC --CCCUCGGU AGUGAAGUUA   858

                 901         911         921         931         941         951         961         971         981         991       1000
                 |           |           |           |           |           |           |           |           |           |         |
CanMyc55   828  ACGCGUUAAA UGCUCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   924
MycoOv13   845  ACGCGTTAAA TGCTCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   941
UncMyc63   754  ACGCGTTAAA TTCTCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   850
CanMy156   845  ACGCGTTAAA TGCTCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   941
CanMy155   845  ACGCGTTAAA TGCTCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   941
MycWeny2   851  ACGCGUUAAA UGCCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   947
UncMyc62   755  ACGCGTTAAA TACCCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   851
MycoSu35   860  ACGCGUUAAA UACCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   956
MycEryth   844  ACGCAUUAAA UGCCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGAUC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   940
CanMyco2   843  ACGCGUUAAA UACCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   939
MycSpe79   842  ACGCGTTAAA TACCCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GAAATT GACGGGGACC TGAACAAGTG GTGGAGCATG TTGCTTAATT   938
MycHaemo   835  ACGCGUUAAA UACCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   931
CanMyco9   850  ACGCGUUAAA UACCCCGCCU GGG-UAGUAU AUAUGCAAAU AUGAAAC-UC AAA-GAAAUU GAUGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   946
CanMyc10   846  ACGCGUUAAA UACCCCGCCU GGGGUAGUAU AUAUGCAAAU AUGAAACCUC AAA-GAAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   944
MycHae90   823  ACGCGUUAAA UACCCCGCCU GGG-UAGUAC AUAUGCAAAU AUGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   919
HmbCanis   808  ACGCGUUAAA UACCCCGCCU GGG-UAGUAC AUAUGCAAAU AUGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   904
CanMyc51   825  ACGUGUUAAA UACCCCGCCU GGG-UAGUAC AUAUGCAAAU AAGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAGCAUG UUGCUUAAUU   921
MycSpe32   805  ACGUGUUAAA UACCCCGCCU GGG-UAGUAC AUAUGCAAAU AUGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAACAUG UUGCUUAAUU   901
UncMyc52   773  ACGTGTTAAA TACCCCGCCT GGG-TAGTAT ATATGCAAAT ATGAAAC-TC AAA-GGAATT GACGGGGACC TGAACAAGTG GTGGAACATG TTGCTTAATT   869
MycCocco   823  ACGUGUUAAA UACCCCGCCU GGG-UAGUAC AUAUGCAAAU AUGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAACAUG UUGCUUAAUU   919
HmbMuris   807  ACGUGUUAAA UAUCCCGCCU GAG-UAGUAC AUAUGCAAAU AUGAAAC-UC AAA-GGAAUU GACGGGGACC UGAACAAGUG GUGGAACAUG UUGCUUAAUU   903
MycCavip   818  ACGCATTAAA TATCCCGCCT GGG-TAGTAC ATTCGCAAGA ATGAAAC-TC AAA-GGAATT GACGGGGACC TGCACAAGTG GTGGAGCATG TTGCTTAATT   914
MycFast3   819  ACGCATTAAA TATCCCGCCT GGG-TAGTAC ACTCGCAAGA GTGAAAC-TC AAA-GGAATT GACGGGGACC TGCACAAGTG GTGGAGCATG TTGCTTAATT   915
MycInso2   825  ACGCATTAAA TATCCCGCCT GGG-TAGTAC ATTCGCAAGA ATGAAAC-TC AAA-GGAATT GACGGGGACC TGCACAAGTG GTGGAGCATG TTGCTTAATT   921
MycPneu5   859  ACACAUUAAG UAUCUCGCCU GGG-UAGUAC AUUCGCAAGA AUGAAAC-UC AAACGGAAUU GACGGGGACC CGCACAAGUG GUGGAGCAUG UUGCUUAAUU   956

                1001        1011        1021        1031        1041        1051        1061        1071        1081        1091      1100
                |           |           |           |           |           |           |           |           |           |         |
CanMyc55   925  CGAUAAUACA CAAAAAACCU UACCAAGGCU UGUUAUCUAC UGCAAAACUA UAGAAAUAUG -GUGG-AGUA U--AUCAGUA AGACAGGUGG UGCAUGGCUG  1020
MycoOv13   942  CGATAATACA CGAAAAACCT TACCAAGGCT TGTTATCTAC TGCAAAACTA TAGAAATATG -GTGG-AGTA T--ATCAGTA AGACAGGTGG TGCATGGCTG  1037
UncMyc63   851  CGATAATACA CGAAAAACCT TACCAAGGCT TGTTATCTAC TGCAAAACTA TAGAAATATA -GTGG-AGTA T--ATCAGTA AGACAGGTGG TGCATGGCTG   946
CanMy156   942  CGATAATACA CGAAAAACCT TACCAAGGCT TGTTATCTAC TGCAAAACTA TAGAAATATA -GTGG-AGGT T--ATCAGTA AGACAGGTGG TGCATGGCTG  1037
CanMy155   942  CGATAATACA CGAAAAACCT TACCAAGGCT TGTTATCTAC TGCAAAACTA TAGAAATATA -GTGG-AGTA T--ATCAGTA AGACAGGTGG TGCATGGCTG  1037
MycWeny2   948  CGAUAAUACA CGAAAAACCU UACCAAGGCU UGCAAUCUAC UGCAAAGCUA UAGAAAUAUA -GUGG-AGGC A--AUCAGUA AGACAGGUGG UGCAUGGCUG  1043
UncMyc62   852  CGATAATACA CGAAAAACCT TACCAAGGCT TGTGATCTCC CGCAAAGCTG TAGAAATATA -GTGG-AGTA T--ATCGGGA TGACAGGTGG TGCATGGCTG   947
MycoSu35   957  CGAUUCUACA CGCAAAACCU UACCAAGGCU UGCAAUCUUC UGCAAAGCUA UAGAAAUAUA -GUGG-AGGC U--AUCAGAA UGACAGGUGG UGCAUGGCUG  1052
MycEryth   941  CGAUAAUACA CGAAAAACCU UACCAAGGCU UGUAAUCUUC UGCAAAAGUG UAGAAAUACA -GUGG-AGUA U--AUCAGAA UGACAGGUGG UGCAUGGCUG  1036
CanMyco2   940  CGAUAAUACA CGAAAAACCU UACCAAGGCU UGUAAUCUUC UGCAAAACUA UAGAAAUAUA -GUAG-AGGC U--AUCAGAA UGACAGGUGG UGCAUGGCUG  1035
MycSpe79   939  CGATAATACA CGCAAAACCT TACCGAGACT TGTAATCTTC TGCGAAGCTA TAGAAATATA -GCGG-AGGC T--ATCGGAA TGACAGGTGG TGCATGGCTG  1034
MycHaemo   932  CGAUAAUACA CGCAAAACCU UACCAAGGCU UGUAAUCUAC UGCGAAACUA UAGAAAUAUA -GUGG-AGGU U--AUCGGUA UGACAGGUGG UGCAUGGCUG  1027
CanMyco9   947  CGAUAAUACA CGAAAAACCU UACCGAGGCU UGUAAUCUUU UGCGAAGCUA UAGAAAUAUA -GUGAA-GGU U--AUCAAAA UGACAGGUGG UGCAUGGCUG  1042
CanMyc10   945  CGAUAAUACA CGAAAAACCU UACCGAGGCU UGUAAUCUUU UGCAAAGCUA UAGAAAUAUA -GUGGA-GGC U--AUCAGAA UGACAGGUGG UGCAUGGCUG  1040
MycHae90   920  CGAUAAUACA CGAAAAACCU UACCAAGGUU UGACAUCCCU CGCAAAGCUA UAGAAAUAUA -GUAGA-G-G UUA-UCGAGG UGACAGGUGG UGCAUGGCUG  1015
HmbCanis   905  CGAUAAUACA CGAAAAACCU UACCAAGGUU UGACAUCCCU CGCAAAGCUA UAGAAAUAUA -GUAGA-G-G UUA-UCGAGG UGACAGGUGG UGCAUGGCUG  1000
CanMyc51   922  CGAUAAUACA CGAAAAACCU UACCAAGGUU UGACAUCCUU CGCAAAGCUA UAGAAAUAUA -GUAGA-G-G UUA-UCGGAG UGACAGGUGG UGCAUGGCUG  1017
MycSpe32   902  CGAUAAUACA CGAAAAACCU UACCAAGGUU UGACAUCCUU UGCAAAGCCA UAGAAAUAUG -GUGG-AG-G UUA-UCAGAG UGACAGGUGG UGCAUGGUUG   997
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Alignment 16S rRNA of haemotrophic mycoplasmas

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UncMyc52   870  CGATAATACA CGAAAAACCT TACCAGGGTT TGACATCCCT TGCGAAACCG TGGAAACACG -GCGG-AG-G TTA-TCAAGG TGACAGGTGG TGCATGGTTG   965
MycCocco   920  CGAUAAUACA CGAAGAACCU UACCAAGGUU UGACAUCCCU CGCAAAACCA UAGAAAUAUG -GCGG-AG-G UUA-UCGAGG UGACAGGUGG UGCAUGGUUG  1015
HmbMuris   904  CGACAAUACA CGAAAAACCU UACCAAGAUU UGACAUCCCC UGCGAAGCUU UAGAAAUAAA -GUGG-AG-G UUA-UCAGGG UGACAGGUGG UGCAUGGCUG   999
MycCavip   915  CGACAATACA CGAAAAACCT TACCCGGGTT TGACATCCCT TGCAAAGCTA TAGAAATATA -GTGG-AG-G CTA-TCAAGG TGACAGGTGG TGCATGGTTG  1010
MycFast3   916  CGACAATACA CGAAAAACCT TACCCAGGTT TGACATCCCT TGCAAAGCTA TGGAAACATA -GTGG-AG-G CTA-TCAAGG TGACAGGTGG TGCATGGTTG  1011
MycInso2   922  CGACAATACA CGAAAAACCT TACCCAGGTT TGACATCCCT TGCAATGTTA TGGAAACATA TACGG-AG-G CTA-TCAAGG TGACAGGTGG TGCATGGTTG  1018
MycPneu5   957  CGACGGUACA CGAAAAACCU UACCUAGACU UGACAUCCUU GGCAAAGUUA UGGAAACAUA -AUGG-AG-G UUA-ACCGAG UGACAGGUGG UGCAUGGUUG  1052

                1101       1111       1121       1131       1141       1151       1161       1171       1181       1191      1200
                |          |          |          |          |          |          |          |          |          |         |
CanMyc55   1021 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCUC UAG-UU-AAU U-AGUUCUAG AGUGACUGA- AUCGUAAGAU  1116
MycoOv13   1038 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTC TAG-TT-AAT T-AGTTCTAG AGTGACTGA- ATCGTAAGAT  1133
UncMyc63    947 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTC TAG-TT-AAT T-AGTTCTAG AGTGACTGA- ATCGTAAGAT  1042
CanMyl56   1038 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTC TAG-TT-AAT T-AGTTCTAG AGTGACTGA- ATCGTAAGAT  1133
CanMyl55   1038 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTC TAG-TT-AAT T-AGTTCTAG AGTGACTGA- ATCGTAAGAT  1133
MycWeny2   1044 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCUC UAG-UU-ACC U-AGUUCUAG AGUGACUGA- AUCGUAAGAU  1139
UncMyc62    948 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTC TAG-TT-ACC T-AGTTCTAG AGTGACTGA- ATCGTAAGAT  1043
MycoSu35   1053 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUUCAUAU UAGUUG-UUU --AGUUCUAA UAUGACUGA- AUCGUAAGAU  1148
MycEryth   1037 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCCUCUCAU UAG-UU-AAA U-AGUUCUAA UGUGACUGA- AUCGUAAGAU  1132
CanMyco2   1036 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCAU UAG-UU-AAU U-AGUUCUAA UGUGACUGA- AUCGUAAGAU  1131
MycSpe79   1035 TCGTCAGCTC GTGTCTTGAG ATGTTTGGTT AAGTCCCGTA ACGAGCGCAA CCCTACTCTT TAG-TT-AAT T-AGTTCTAA AGTGACTGA- ATCGTAAGAT  1130
MycHaemo   1028 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCUC UAG-UU-AAU U-AGUUCUAG AGUGACUGA- AUCGUAAGAU  1123
CanMyco9   1043 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCUU UAG-UU-AAU U-AGUUCUAA AGUGACUGA- AUCGUAAGAU  1138
CanMyc10   1041 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGUA ACGAGCGCAA CCCUACUCUU UAG-UU-AAC C-AGUUCUAA AGUGACUGA- AUCGUAAGAU  1136
MycHae90   1016 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGCA ACGAGCGCAA CCCCACUCUU UAG-UUA--C -UUG-UCUAA AGAGACUGC- ACAGUAAUGU  1109
HmbCanis   1001 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGCA ACGAGCGCAA CCCCACUCUU UAG-UUA--C -UUG-UCUAA AGAGACUGC- ACAGUAAUGU  1094
CanMyc51   1018 UCGUCAGCUC GUGUCUUGAG AUGUUUGGUU AAGUCCCGCA ACGAGCGCAA CCCUACUCUU UAG-UUA--U -UUU-UCUAA AGAGACUGC- ACAGUAAUGU  1111
MycSpe32    998 UCGUCAGCUC GUGUCAUGAG AUGUUUGGUU AAGUCCCGCA ACGAGCGCAA CCCUACUCUU UAG-UUG--A -UUG-UCUAA AGAGACUGA- ACAGUAAUGU  1091
UncMyc52    966 TCGTCAGCTC GTGTCATGAG ATGTTTGGTT AAGTCCCGCA ACGAGCGCAA CCCTACCCCT TAG-TTG--T -TTT-TCTAA GGAGACTGC- ACAGTAATGT  1059
MycCocco   1016 UCGUCAGCUC GUGUCAUGAG AUGUUUGGUU AAGUCCCGCA ACGAGCGCAA CCCUACUCUU UAG-UUA--C UUUA-UCUAA AGAGACUGA- ACAGUAAUGU  1110
HmbMuris   1000 UCGUCAGCUC GUGUCAUGAG AUGUCUGGUU AAGUCCUGAA ACGAGCGCAA CCCUACUCUU UAG-UUA--A -CUU-UCUAA AGAGACUGA- ACAGUAAUGU  1093
MycCavip   1011 TCGTCAGCTC GTGTCGTGAG ATGTTTGGTT AAGTCCCGCA ACGAGCGCAA CCCCTGTTTG TAG-TTA--A -GTG-TCTAC AAAGACTGA- AGCGAAAGCT  1104
MycFast3   1012 TCGTCAGCTC GTGTCGTGAG ATGTTTGGTT AAGTCCCGCA ACGAGCGCAA CCCCTGTCTG TAG-TTA--A -GTA-TCTAC AGAGACTGA- AGCGAAAGCT  1105
MycInso2   1019 TCGTCAGCTC GTGTCGTGAG ATGTTTGGTT AAGTCCCGCA ACGAGCGCAA CCCCTATCTT TAG-TTA--T -GTG-TCTAA AGAGACTGA- AGCGAAAGCT  1112
MycPneu5   1053 UCGUCAGCUC GUGUCGUGAG AUGUUGGGUU AAGUCCCGCA ACGAGCGCAA CCCUUAUCGU UAG-UUA--C AUUG-UCUAG CGAGACUGCU AAUGCAAAUU  1148

                1201       1211       1221       1231       1241       1251       1261       1271       1281       1291      1300
                |          |          |          |          |          |          |          |          |          |         |
CanMyc55   1117 -AUAGGAAGG AUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG UUGCAAACGU GCUACAAUGG UAGGUACAAU GUGU-UGCAA UCUAGCGAUA  1214
MycoOv13   1134 -ATAGGAAGG ATGGGGCCAA GTCAAGTCAT CATGCCCCTT ATGCCTTGGG TTGCAAACGT GCTACAATGG TAGGTACAAT GTGT-TGCAA TCTAGCGATA  1231
UncMyc63   1043 -ATAGGAAGG ATGGGGCCAA GTCAAGTCAT CATGCCCCTT ATGCCTTGGG CTGCAAACGT GCTACAATGG TAGGTACAAT GTGC-TGCAA TCTAGCGATA  1140
CanMyl56   1134 -ATAGGAAGG ATGGGGCCAA GTCAAGTCAT CATGCCCCTT ATGCCTTGGG CTGCAAACGT GCTACAATGG TAGGTACAAT GTGT-TGCAA ACTAGCGATA  1231
CanMyl55   1134 -ATAGGAAGG ATGGGGCCAG GTCAAGTCAT CATGCCCCTT ATGCCTTGGG CTGCAAACGT GCTACAATGG TAGGTACAAT GTGT-TGCAA TCTAGCGATA  1231
MycWeny2   1140 -AUAGGAAGG UUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG UAGGUACAAU GUGU-UGCAA ACUAGCGAUA  1237
UncMyc62   1044 -ATAGGAAGG TTGGGGCCAA GTCAAGTCAT CATGCCCCTT ATGCCTTGGG CTGCAAACGT GCTACAATGG TAGGTACAAT GTGT-AGCAA GCTAGCGATA  1141
MycoSu35   1149 -CUAGGAAGG AUGGGGCCAA GUUAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG UAGAUACAAU GUGU-UACAA UCUAGCGAUA  1246
MycEryth   1133 -AUAGGAAGG AUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG UAGGUACAAU GUGU-UGCAA CUUAGCGAUA  1230
CanMyco2   1132 -AUAGGAAGG AUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG UAGGUACAAU GUGU-UGCAA CCUAGUAAUA  1229
MycSpe79   1131 -ATAGGAAGG TTGGGGCCAA GTCAAGTCAT CATGCCCCTT ATGTCTCGGG CTGCAAACGT GCTACAATGG TAGGTACAAT GTGT-TGCAA TCTAGCGATA  1228
MycHaemo   1124 -AUAGGAAGG UUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG UAGGCACAAU GUGU-UGCAA ACUAGCGAUA  1221
CanMyco9   1139 -AUAGGAAGG CUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUCGGG CUGCAAACGU GCUACAAUGG UAGGCAUAAU GUGU-UGCAA ACUAGAAAUA  1236
CanMyc10   1137 -AUAGGAAGG UUGGGGCCAA GUCAAGUCAU CAUGCCCCUU AUGCCUCGGG CUGCAAACGU GCUACAAUGG UAGGUACAAU GUGU-UGCAA UCUAGCAAUA  1234
MycHae90   1110 -AGAGGAAGG AUGGGAUCAC GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG CGAACACAAU GUGU-UGCAA ACCAGCGAUG  1207
HmbCanis   1095 -AGAGGAAGG AUGGGAUCAC GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG CGAACACAAU GUGU-UGCAA ACCAGCGAUG  1192
CanMyc51   1112 -AGAGGAAGG UUGGGAUCAC GUCAAGUCAU CAUGCCCCUU AUGCCUUGGG CUGCAAACGU GCUACAAUGG CGAACACAAU GUGU-UGCAA AUCAGCGAUG  1209
MycSpe32   1092 -AUAGGAAGG AUGGGAUCAC GUCAAAUCAU CAUGCCCCUU AUGCCUUGGG CCGCAAACGU GUUACAAUGG UGAGUACAAU GUGU-CGCGA ACCAGCGAUG  1189
UncMyc52   1060 -AGAGGAAGG ATGGGATCAC GTCAAATCAT CATGCCCCTT ATGCCCTGGG CTGCAAACGT GTTACAATGG TGGATACAAT ATGTCTGCAA ACCAGCGATG  1158
MycCocco   1111 -AUAGGAAGG AUGGGAUCAC GUCAAAUCAU CAUGCCCCUU AUGCCUUGGG CCGCAAACGU GUUACAAUGG GAGGUACAAU GUGU-CGCAA ACUAGCGAUA  1208
HmbMuris   1094 -AUAGGAAGG AUGGGAUCAC GUCAAGUCAU CAUGCCCCUU AUAUCUUGGG CCGCAAACGU GUUACAAUGG UGGGUACAAC GUGU-CGCAA GCCAGCGAUG  1191
MycCavip   1105 -ATAGGAAGG TGGGGATGAC GTCAAATCAT CATGCCCCTT ATGTCCGGGG CTGCAAACGT GCTACAATGG TTGATACAAA GTG--GGCAA TACAGTGATG  1201
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Alignment 16S rRNA of haemotrophic mycoplasmas

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MycFast3  1106  -ATAGGAAGG TGGGGATGAC GTCAAATCAT CATGCCCCTT ATGCCTGGGG CTGCAAACGT GCTACAATGG TTGGTACAAA GTG--GGCGA TACAGCGATG  1202
MycInso2  1113  -ATAGGAAGG TGGGGATGAC GTCAAATCAT CATGCCCCTT ATGCCTGGGG CTGCAAACGT GCTACAATGG TTGGTACAAA GTG--TGCAA TGCAGCAATG  1209
MycPneu5  1149  G-GAGGAAGG AAGGGAUGAC GUCAAAUCAU CAUGCCCCUU AUGUCUAGGG CUGCAAACGU GCUACAAUGG CCAAUACAAA CAGU-CGCCA GCUUGUAAAA  1246

                1301       1311       1321       1331       1341       1351       1361       1371       1381       1391      1400
                |          |          |          |          |          |          |          |          |          |         |
CanMyc55  1215  GUGAGCU-AA UCACCGAAA- ACCUAUCUCA GUCCGGAUAA AAGGUUGCAA UUCGCCUAUU UGAAGUUGGA AUUACUAGUA AUCCUGUGUC AGCUAUAUCA  1312
MycoOv13  1232  GTGAGCT-AA TCACCGAAA- ACCTATCTCA GTCCGGATAA AAGGTTGCAA TTCGCCTATT TGAAGTTGGA ATTACTAGTA ATCCTGTGTC AGCTATATCA  1329
UncMyc63  1141  GTGAGCT-AA TCACCGAAA- ACCTATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTATT TGAAGTTGGA ATCACTAGTA ATCCTGTGTC AGCTATATCA  1238
CanMy156  1232  GTGAGCT-AA TCACCTAAA- GCCTATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTATT TGAAGTTGGA ATCACTAGTA ATCCTGTGTC AGCTATATCA  1329
CanMy155  1232  GTGAGCT-AA TCACCGAAA- ACCTATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTATT TGAAGTTGGA ATCACTAGTA ATCCTGTGTC AGCTATATCA  1329
MycWeny2  1238  GUGAGCU-AA UCACCUAAA- GCCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGUUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1335
UncMyc62  1142  GTGAGCA-AA TCACCGAAA- GCCTATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTATT TGAAGTTGGA ATCACTAGTA ATCCTGTGTC AGCTATATCA  1239
MycoSu35  1247  GUGAGUU-AA UCACCUAAA- GUCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGAUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1344
MycEryth  1231  AUGAGCU-AA UCACCGAAA- ACCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGAUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1328
CanMyco2  1230  GGAAGCC-AA UCAC-UAAA- UCCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGUUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1326
MycSpe79  1229  GTGAGCT-AA TCACCGAAA- ACCTATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTATT TGAAGTTGGA ATCACTAGTA ATCCTGTGTC AGCTATATCA  1326
MycHaemo  1222  GUGAGCU-AA UCACCUAAA- ACCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGAUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1319
CanMyco9  1237  GGGAGCU-AA UCACCGAAA- ACCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGUUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1334
CanMyc10  1235  GUGAGCA-AA UCACCCAAA- ACCUAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUAUU UGAAGUUGGA AUCACUAGUA AUCCUGUGUC AGCUAUAUCA  1332
MycHae90  1208  GUAAGCU-AA UCACC-AAA- UUUCGUCUCA GUUCGGAUAG GAGGCUGCAA UUCGCCUCCU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCUAUAUCG  1304
HmbCanis  1193  GUAAGCU-AA UCACC-AAA- UUUCGUCUCA GUUCGGAUAG GAGGCUGCAA UUCGCCUCCU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCUAUAUCG  1289
CanMyc51  1210  AUGAGCU-AA UCACU-AAA- UUUUGUCUCA GUUCGGAUAG AAGGCUGCAA UUCGCCUUCU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCUAUAUCG  1306
MycSpe32  1190  GUAAGCU-AA UCAC-CAAA- ACUCAUCUCA GUCCGGAUAA AAGGCUGCAA UUCGCCUUUU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCUAUAUCG  1286
UncMyc52  1159  GTAAGCT-AA TCAT-TAAA- ATTCATCTCA GTCCGGATAA AAGGCTGCAA TTCGCCTTTT TGAAGTTGGA ATCACTAGTA ATCCCGTGTC AGCTATATCG  1255
MycCocco  1209  GUAAGCU-AA UCAC-UAAA- GCCUCUCCCA GUUCGGAUAA AAGGCUGCAA UUCGCCUUUU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCUAUAUCG  1305
HmbMuris  1192  GCAAGCC-AA UCAC-UAAAA GCCCAUCCCA GUCCGGAUAA AAGGCUGCAA UUCGCCUUUU UGAAGUUGGA AUCACUAGUA AUCCCGUGUC AGCCAUAUCG  1289
MycCavip  1202  TAAAGCATAA TCACA-AAA- GTCAATCTCA GTTCGGATTG AAGGCTGCAA CTCGCCTTCA TGAAGTTGGA ATCACTAGTA ATCGCGTGTC AGCTATATCG  1299
MycFast3  1203  TAAAGCTTAA TCACA-AAA- GCCAATCTCA GTTCGGATTG AAGGCTGCAA CTCGCCTTCA TGAAGTTGGA ATCACTAGTA ATCGCGTGTC AGCTATATCG  1300
MycInso2  1210  CAAAGCTTAA TCACA-AAA- GCCAATCTCA GTTCGGATTG AAGGCTGCAA CTCGCCTTCA TGAAGTTGGA ATCACTAGTA ATCGCGTGTC AGCTATATCG  1307
MycPneu5  1247  GUGAGCA-AA UCUG-UAAA- GUUGGUCUCA GUUCGGAUUG AGGGCUGCAA UUCGUCCUCA UGAAGUCGGA AUCACUAGUA AUCGCGAAUC AGCUAUGUCG  1343

                1401       1411       1421       1431       1441       1451       1461       1471       1481       1491      1500
                |          |          |          |          |          |          |          |          |          |         |
CanMyc55  1313  GGGUGAAAAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GAAA-GUAUC AGUCAAAACC GCA-U----- ---UCAA--- -----UUGUG  1394
MycoOv13  1330  GGGTGAATAC GTTCCCAGGT CTTGTACACA CCGCCCGTCA AACTATGAAA GAAA-GTATC AGTCAAAACC GCA-T----- ---TCAA--- -----TTGTG  1411
UncMyc63  1239  GGG-GAATAC GT-CCCA... .......... .......... .......... .......... .......... .......... .......... ..........  1253
CanMy156  1330  GGGTGAATAC GTTCCCAGGT CTTGTACACA CCGCCCGTCA AACTATGAAA GGAA-GTACT AGTCAAAACC GCA-T----- ---TAAA--- -----TTGTG  1411
CanMy155  1330  GGGTGAATAC GTTCCCAGGT CTTGTACACA CCGCCCGTCA AACTATGAAA GAAA-GTACT AGTCAAAACC GTA-T----- ---TAAA--- -----TTACG  1411
MycWeny2  1336  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GAAA-GUACU AGUCAAAACC ACA-U----- ---UCAA--- -----UUGUG  1417
UncMyc62  1240  GGGTGAATAC GTC....... .......... .......... .......... .......... .......... .......... .......... ..........  1252
MycoSu35  1345  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUACGAAA GAAA-GUACU AAUUAAAACC GUA-U----- ---UUAA--- -----UUACG  1426
MycEryth  1329  GGGUGAAUGC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GAAA-GUACU AGUUAAAACC GCA-U----- ---UUAA--- -----UUGCG  1410
CanMyco2  1327  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUACGAAA GAAU-GUACU AGUUGAAACC GCA-U----- ---UUAA--- -----UUGUG  1408
MycSpe79  1327  GGGTGAATAC GTTCCCAGGT CTTGTACACA CCGCCCGTCA AACTATGAAA GAAA-GTACT AGTTGAAACC GCA-T----- ---TTAG--- -----TTGCG  1408
MycHaemo  1320  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GAAA-GAACU AGUUGAAACC GCA-U----- ---UUAA--- -----UUGCG  1401
CanMyco9  1335  GGGUGAAUGC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGGGA GAAA-GUACU AGUUGAAACC GUA-U----- ---UUAA--- -----UUACG  1416
CanMyc10  1333  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GAAACGUACU AGUUGAAACC GUA-U----- ---UUAA--- -----UUACG  1415
MycHae90  1305  GGGUGAAUCC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA GGAG-UGGGC AUUUAAAAAU ACA-U----- ---UCAU--- -----UUGUA  1386
HmbCanis  1290  GGGUGAAUCC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA GGAG-UGGGC AUUUAAAAAU ACA-U----- ---UUAU--- -----UUGUA  1371
CanMyc51  1307  GGGUGAAUGC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA GGAG-UGGGC AUUUAAAAAU GUA-U----- ---UCAU--- -----UUGCA  1388
MycSpe32  1287  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA GGAA-GGGGC AUUUGAAAAC ACA-U----- ---UCAA--- -----UUGUG  1368
UncMyc52  1256  GGGTGAATGC GTTCCCAGGT CTTGTACACA CCGCCCGTCA AACTATGAGA GGAA-GGGGC GTTTAAAAAT ACA-T----- ---TTAT--- -----TTGTA  1337
MycCocco  1306  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA G......... .......... .......... .......... ..........  1356
HmbMuris  1290  GGGUGAAUAC GUUCCCAGGU CUUGUACACA CCGCCCGUCA AACUAUGAGA GGGA-GAGGC AUUCGAAAAC GCA-U----- ---UCAU--- -----UUGCG  1371
MycCavip  1300  CGGTGAATAC GTTCTCAGGT CTTGTACACA CCGCCCGTCA AACTACGAGA GGTA-AGTAT ATCTAAAACC GCAAAATTAA CCTGCAAAGT GGAATATGCG  1398
MycFast3  1301  CGGTGAATAC GTTCTCAGGT CTTGTACACA CCGCCCGTCA AACTACGAGA GGTA-AGTAT ATCTAAAACC GCAAAATTAA CCTGCAAAGT GGAATATGCG  1399
MycInso2  1308  CGGTGAATAC GTTCTCAGGT CTTGTACACA CCGCCCGTCA AACTACGAGA GGTA-AGTAT GTCTAAAACC GCAAAATTAA CCTGCAAAGT GGAATATGCG  1406
MycPneu5  1344  CGGUGAAUAC GUUCUCGGGU CUUGUACACA CCGCCCGUCA AACUAUGAAA GCUG-GUAAU AUUUAAAAAC GUGUUGCUAA CC-AUUA-G- GAAGCGCAUG  1439
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Alignment 16S rRNA of haemotrophic mycoplasmas

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                1501       1511       1521       1531       1541       1551       1561       1571       1581       1591      1600
                |          |          |          |          |          |          |          |          |          |         |
CanMyc55  1395  UCUAGAUUGG UAAUUUUGAU UGGAGUUAAG UCGUAACAAG GUAACCG... .......... .......... .......... .......... ..........  1441
MycoOv13  1412  TCTAGATTGG TAATTTTGAT TGGAGTTAAG TCGTAACAAG GTAACC.... .......... .......... .......... .......... ..........  1457
UncMyc63  1254  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1253
CanMy156  1412  TCTAGATTGG TAATTCTGAT TGG....... .......... .......... .......... .......... .......... .......... ..........  1434
CanMy155  1412  TCTAGATTGG TAATTTTGAT TGG....... .......... .......... .......... .......... .......... .......... ..........  1434
MycWeny2  1418  UCUAGAUUGG UAAUUUUGAU UGGAGUUAAG UCGUAACAAG GUAGCGGAUC CGCG..... .......... .......... .......... ..........  1471
UncMyc62  1253  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1252
MycoSu35  1427  UCUAGAUUGG UAAUUUUGAU UGGAGUUAAG UCGUAACAAG GUA....... .......... .......... .......... .......... ..........  1469
MycEryth  1411  UCUAGAUUGG UAAUUUUAAU UGGAGUUAAG UCGUAACAAG GUA....... .......... .......... .......... .......... ..........  1453
CanMyco2  1409  UC........ .......... .......... .......... .......... .......... .......... .......... .......... ..........  1410
MycSpe79  1409  TCTAGATTGG TAATTTTGAT TGGAGTTAAG .......... .......... .......... .......... .......... .......... ..........  1438
MycHaemo  1402  UCUAGAUUGG UAAUUUUGAU UGGAGUU... .......... .......... .......... .......... .......... .......... ..........  1428
CanMyco9  1417  UCUAGAUUGG UAAUUUUGAC UGGAGUUAAG UCGUAACAAG GUAGCGGAUC CGCG..... .......... .......... .......... ..........  1470
CanMyc10  1416  UCUAGAUUGG UAAUUUUGAU UGGAGUUAAG UCGUAACAAG GUAACC.... .......... .......... .......... .......... ..........  1461
MycHae90  1387  UCUAGAGUGA ACAUUCUGAU UGGAGUUAAG UCGUAACAAG GUA....... .......... .......... .......... .......... ..........  1429
HmbCanis  1372  UCUAGAGUGA ACAU-CUGAU UGGAGUU... .......... .......... .......... .......... .......... .......... ..........  1397
CanMyc51  1389  UCUAGAGUGA ACAUUCUGAG UGGAGUUAAG UCGUAACAAG GUAGCCG... .......... .......... .......... .......... ..........  1435
MycSpe32  1369  UCAAGAAUGA AACUUCUGAU UGGAGUU... .......... .......... .......... .......... .......... .......... ..........  1395
UncMyc52  1338  TCTAGAACGA .......... .......... .......... .......... .......... .......... .......... .......... ..........  1347
MycCocco  1357  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1356
HmbMuris  1372  UCUAGAAUGA AUUUUCCGAU UGGAGUUAAG .......... .......... .......... .......... .......... .......... ..........  1401
MycCavip  1399  TCTAGGATAG ATTTACTGAT TGGAGTTAAG TCGTAACAAG GTACCC.... .......... .......... .......... .......... ..........  1444
MycFast3  1400  TCTAGGATAG ATTTGCTGAT TGGAGTTAAG TCGTAACAAG GTACCCCTAC GAGAAC.... .......... .......... .......... ..........  1455
MycInso2  1407  TCTAGGATAG ATTTACTGAT TGGAGTTAAG TCGTAACAAG GTACCCCTAC GAGAACGTGG GGGTGGATCA CCTCCTTTCT TTGGAATTAA AGTTTTTAAC  1506
MycPneu5  1440  UCAAGGAUAG CACCGGUGAU UGGAGUUAAG UCGUAACAAG GUACCCCUAC GAGAACGUGG GGGUGGAUCA CCU....... .......... ..........  1512

                1601       1611       1621       1631
                |          |          |          |
CanMyc55  1442  .......... .......... .......... ..........                                                              1441
MycoOv13  1458  .......... .......... .......... ..........                                                              1457
UncMyc63  1254  .......... .......... .......... ..........                                                              1253
CanMy156  1435  .......... .......... .......... ..........                                                              1434
CanMy155  1435  .......... .......... .......... ..........                                                              1434
MycWeny2  1472  .......... .......... .......... ..........                                                              1471
UncMyc62  1253  .......... .......... .......... ..........                                                              1252
MycoSu35  1470  .......... .......... .......... ..........                                                              1469
MycEryth  1454  .......... .......... .......... ..........                                                              1453
CanMyco2  1411  .......... .......... .......... ..........                                                              1410
MycSpe79  1439  .......... .......... .......... ..........                                                              1438
MycHaemo  1429  .......... .......... .......... ..........                                                              1428
CanMyco9  1471  .......... .......... .......... ..........                                                              1470
CanMyc10  1462  .......... .......... .......... ..........                                                              1461
MycHae90  1430  .......... .......... .......... ..........                                                              1429
HmbCanis  1398  .......... .......... .......... ..........                                                              1397
CanMyc51  1436  .......... .......... .......... ..........                                                              1435
MycSpe32  1396  .......... .......... .......... ..........                                                              1395
UncMyc52  1348  .......... .......... .......... ..........                                                              1347
MycCocco  1357  .......... .......... .......... ..........                                                              1356
HmbMuris  1402  .......... .......... .......... ..........                                                              1401
MycCavip  1445  .......... .......... .......... ..........                                                              1444
MycFast3  1456  .......... .......... .......... ..........                                                              1455
MycInso2  1507  TTTTTAGTTA AAAAATTAAT ACAAATATAG TGTACTTTTT                                                              1546
MycPneu5  1513  .......... .......... .......... ..........                                                              1512
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Alignment 16S rRNA of bovine HM

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                1          11         21         31         41         51         61         71         81         91        100
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis      1 ........AG AGUUUGAUCC UGGCUCAGGA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA GUAG--AACU U-GUU--CUG CUAGUGGCAA     87
MycWenyo      1 .......... .......... .......... .UAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA GUGG--AACU U-GUU--CUG CUAGUGGCGA     64
MycWeny8      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
MycWeny9      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
EperSuis      1 .......... .......... .......... .......... .......... .......... .........A AAAAGGCCCU C-GGGUCUUU UUAGUGGCAA     30
MycEryth      1 ........AG AGUUUGAUCC UGGCUCAGGA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA UUUU-UAAGC A-AUUA-AAG AUAGUGGCGA     89
HmbFeli3      1 ........AG A-UUUGAUCC UGGCUCAGAA UUAAUGCUGA UGGUAUGCCU AAUACAUGCA AGUCGAACGG AUCUUGGUUU C-GGCCAAGA UUAGUGGCAA     90
HmbCanis      1 .......... .......... ...CUCAGAA UUAAUGCUGA UGGUAUGCCU AAUACAUGCA AGUCGAACGG ACCUUGGUUU C-GGCCAAGG UUAGUGGCAA     76
MycSpe66      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
MycSpe68      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
MycSpe67      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      0
CanMyc51      1 ........AG AGUUUGAUCC UGGCUCAGAA UUAAUGCUGA UGGUAUGCCU AAUACAUGCA AGUCGAACGG ACUUUGGUUU C-GGCCAAAG UUAGUGGCGA     91
CanMyc44      1 .......... .......... .......... .......... .......... .......... ........GA ACUG--UCCA AAAGG--CAG UUAGCGGCGA     28
HmbMuris      1 .......... .......... ...CUCAGAA UUAACGCUGA UGGCAUACCU AAUACAUGCA AGUCGAGCGG ACCU-CUAGC A-AUAG-AGG UUAGCGGCGA     74
MycPneu5      1 UUUUUCUGAG AGUUUGAUCC UGGCUCAGGA UUAACGCUGG CGGCAUGCCU AAUACAUGCA AGUCGAUCGA AAGU---AGU A-AU---ACU UUAGAGGCGA     93

                101        111        121        131        141        151        161        171        181        191       200
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis     88 ACGGGCGAGU AAUGCAUAUU UAACUUACUU UCGCGAGGAG GAUAGCAGCC CGAAAGGGCU AUUAAUACUA CAUAG-GUUU AUGG------ --AC----UU   174
MycWenyo     65 ACGGGCGAGU AAUACAUAUU UAACUUACUU UUACGAGGAG GAUAGCAGCU CGAAAGGGCU AUUAAUACUC CAUAG-GUUU A--------U AAAC------   149
MycWeny8      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycWeny9      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
EperSuis     31 ACGGGCGAGU AACGCAUACU UAACUUACUU AUCUGAGGAA AAUAGCAGCU CGAAAGAGCU AUUAAUAAUC CAUAG-GUUU AGGC-UAGAG G-AA-CUAGC   126
MycEryth     90 ACGGGCGAGU AAUACAUAUC UAACUUACUU AUGUGAGGGG AAUAGCAGCC CGAAAGGGCU AUUAAUGCUC CAUAG-GUUU A----U---- A-GA---A--   174
HmbFeli3     91 ACGGGUGAGU AAUACAUAUC UAACAUGCCC CUCUGUGGGG GAUAGCCGCU UGAAAAAGCG AUUAAUACCC CAUAG-GAAG CUUU-A-UC- --UAUGAUUU   184
HmbCanis     77 ACGGGUGAGU AAUACAUAUC UAACAUGCCC CUCUGUGGGG GAUAGCCACU UGAAAAAGUG AUUAAUACCC CAUAG-GAAG CUUU-A-UC- --CAUGAUUU   170
MycSpe66      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycSpe68      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycSpe67      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
CanMyc51     92 ACGGGUGAGU AAUACAUAUC UAACAUGCCC CUCUGUAGGG AAUAGCCACU UGAAAAGGUA AUUAAUACCC UAUAG-GUAA CUUU-C-UC- A-CAAGAGUU   186
CanMyc44     29 ACGGGUGAGU AAUACAUAUU UAACAUGCCC UCCGGAAGGA AAUAGCCGUU CGAAAGAACG AUUAAUGUCC UAUAG-UAUC CUCC-A-UCA G-ACAGAAGG   124
HmbMuris     75 ACGGGUGAGU AAUGAAUACU UAACAUACCU CCAUGAAGGA AAUAGCUAUU CGAAAGAGUA AUUAAUGUCC UAUAG-GAGC CUUCCU-CA- --C-AUGAGG   168
MycPneu5     94 ACGGGUGAGU AACACGUAUC CAAUCUACCU UAUAAUGGGG GAUAACUAGU UGAAAGACUA GCUAAUACCG CAUAAGAACU UUGG-U-UC- G-CAUGAAUC   189

                201        211        221        231        241        251        261        271        281        291       300
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis    175 -GUAAAUUAA AGGAGGCGCC C--UC-GGGA GCCUCGCGCG GAAAAGGGAA UAUGUCCUAU UAGGUAGUUG GCGGGGUAAA GGCCCACCAA GCCAAUGAUG   270
MycWenyo    150 --UAAAUUAA A-GAGGCUCC U--C--UGGG GCCUUGCGUA AAACUAGGAA UAUGUCCUAU UAGGUAGUUG GCGGGGUAAA GGCCCACCAA GCCAAUGAUG   242
MycWeny8      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycWeny9      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
EperSuis    127 -UUAAAUUAA AGGAGGCUGC CG-CAAGGUG GCCUUGCGGG UAAAUAGGAG UAUGUCCUAU UAGAUAGUUG GAGAGGUAAA GGCUCACCAA GUCGAUGAUG   224
MycEryth    175 --UAAAUUAA AUGAGGCUC- CG-CAAG-GG GCCUCGCGCA UAAACAGGGA UAUGUCCUAU UAGGUAGUUG GUGGGGUAAA AGCCUACCAA GCCUGUGAUG   269
HmbFeli3    185 -AGCUUUUAA A-GC-CU--- ---UC----- GG-GCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUAGUUG GCGGGAUAAA AGCCCACCAA GGCAAUGAUA   269
HmbCanis    171 -AGCUUUUAA A-GC-CU--- ---UC----- GG-GCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUAGUUG GCGGGAUAAA AGCCCACCAA GGCAAUGAUA   255
MycSpe66      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycSpe68      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycSpe67      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
CanMyc51    187 -AGUUAUUAA A-GC-U---- --UUA----- -U-GCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUUGUUG GCGGGGUAAA AGCCCACCAA GGCUAUGAUA   270
CanMyc44    125 -GGGAUUUAA A-GG-UG--- ---AA----- AA-CCGCCGG AGGAUUGGAA UAUGUCCUAU UAGCUAGUUG GCGGGAUAAA AGCCCACCAA GGCGAUGAUA   209
HmbMuris    169 UUGGCUUUAA A-GG-CG--- ---CA----- AG-CCACUUG GAGAUUGGAG UAUUUUCUAU UAGCUAGUUG GCGGGAUAAU AGCCCACCAA GCGAGUGAUA   254
MycPneu5    190 -AAAGUUGAA A-GGACCUG- ---CA---AG GGUUCGUUAU UUGAUGAGGG UGCGCCAUAU CAGCUAGUUG GUGGGGUAAC GGCCUACCAA GGCAAUGACG   280

                301        311        321        331        341        351        361        371        381        391       400
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis    271 GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   370
MycWenyo    243 GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   342
MycWeny8      1 .......... .......... ...CAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG    77
MycWenv9      1 .......... .......... .......... .......... ......GGCC CAUAUUCCUG CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG    54
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Alignment 16S rRNA of bovine HM

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EperSuis   225  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   324
MycEryth   270  GGUAGCUGGA CUGAGAGGUU GACCAGCCGC AAUGGGAUUG AGAGAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   369
HmbFeli3   270  GAUAGCUGGU CUUAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   369
HmbCanis   256  GAUAGCUGGU CUUAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   355
MycSpe66     1  .......... .......... .......... .......... ......GGCC CAUAUUCCUG CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG    54
MycSpe68     1  .......... .......... .......... .......... ......GGCC CAUAUUCCUG CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG    54
MycSpe67     1  .......... .......... .......... .......... ......GGCC CAUAUUCCUG CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG    54
CanMyc51   271  GAUAGCUGGU CAUAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   370
CanMyc44   210  GGUAGCUGGU CUAAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGGCGAAAG   309
HmbMuris   255  GAUAGCUGGU CUAAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGGCGAAAG   354
MycPneu5   281  UGUAGCUAUG CUGAGAAGUA GAAUAGCCAC AAUGGGACUG AGACACGGCC CAUACUCCUA CGGGAGGCAG CAGUAGGGAA UUUUUCACAA UGAGCGAAAG   380

                401        411        421        431        441        451        461        471        481        491       500
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis   371  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUAUU UAGGAAAAA- AAGCGCGC-- -UAGGAAAUG A---GCGCGC   462
MycWenyo   343  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUAUU UAGGAAAAA- AAGCGUGC-- -UAGGAAAUG A---GCACGC   434
MycWeny8    78  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUAUU UAGGAAAAA- AAGCGUGC-- -UAGGAAAUG A---GCGCGC   169
MycWeny9    55  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUAUU UAGGAAAAA- AAGCGUGC-- -UGGGAAAUG A---GCGCGC   146
EperSuis   325  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUAUU UAGGAAAAA- AAGCGCGA-- -CAGGAAAUG G---UCGCGC   416
MycEryth   370  UCUGAUGGAG CAAUACCACG UGAACGAUGA AGGUCUU-CU GAUUGUAAAG UUCUUUUACU UAGGAAAAA- AAGCUUGA-- -CAGGAAAUG G---UUAAGC   461
HmbFeli3   370  UCUGAUGGAG CAAUACCAUG UGAACGAUGA AGGCCUUUUU GGUUGUAAAG UUCUUUUACG AGGGAUA--- ---------- ----AUUAUG A---------   443
HmbCanis   356  UCUGAUGGAG CAAUACCAUG UGAACGAUGA AGGCCUUUUU GGUUGUAAAG UUCUUUUACG AGGGAUA--- ---------- ----AUUAUG A---------   429
MycSpe66    55  UCUGAUGGAG CAAUACCAUG UGAACGAUGA AGGUCUUUUU GAUUGUAAAG UUCUUUUAUG AGGGAUA--- ---------- ----ACAACU G---------   128
MycSpe68    55  UCUGGUGGAG CAAUACCAUG UGAACGAUGA AGGUCUUUUU GAUUGUAAAG UUCUUUUAUG AGGGAUA--- ---------- ----ACAACU G---------   128
MycSpe67    55  UCUGAUGGAG CAAUACCAUG UGAACGAUGA AGGUCUUUUU GAUUGUAAAG UUCUUUUAUG AGGGAUA--- ---------- ----ACAACU G---------   128
CanMyc51   371  UCUGAUGGAG CAAUACCAUG UGAACGAUGA AGGUCUUUUU GAUUGUAAAG UUCUUUUAUG AGGGAUA--- ---------- ----ACAACU G---------   444
CanMyc44   310  CCUGAUGGAG CAAUGCCAUG UGAACGAUGA AGGCCAGACA GGUCGUAAAG UUCUUUUAGA GGGGAAA--- ---------- ----AAUUUG A---------   383
HmbMuris   355  CCUGAUGGAG UGAUGCCAUG UGAACGAUGA AGGUCUUUUU GAUUGUAAAG UUCUUUUAUU GGGGAAA--- ---------- ----AUGAUG A---------   428
MycPneu5   381  CUUGAUGGAG CAAUGCCGCG UGAACGAUGA AGGUCUUUAA GAUUGUAAAG UUCUUUUAUU UGGGAAGAAU GACUUUAGCA G---GU-AA- -UGGCUAGAG   474

                501        511        521        531        541        551        561        571        581        591       600
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis   463  CUUGAUGGUA CUA-AUUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAAAAC AUAGGUCACA AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   561
MycWenyo   435  CUUGAUGGUA CUA-AUUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAAAAC AUAGGUCACG AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   533
MycWeny8   170  CUUGAUGGUA CUA-AUUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAAAAC AUAGGUCACG AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   268
MycWeny9   147  CUUGAUGGUA CUA-AUUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAAAAC AUAGGUCACG AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   245
EperSuis   417  CCUGAUUGUA CUA-AUUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAAAAC AUAGGUCACG AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   515
MycEryth   462  CUUGAUCGUA CUA-GGUGAA UAAGUGACAG CUAACUAUGU GCCAGCAGCU GCGGUAACAC AUAGGUCACA AGCAUUAUCC GGAUUUAUUG GGCGUAAAGG   560
HmbFeli3   444  -----UAGUA CUU-GCUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCG AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   537
HmbCanis   430  -----UAGUA CUU-CGUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCA AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   523
MycSpe66   129  ----AUAGUA CCU-CAUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCA AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   223
MycSpe68   129  ----AUAGUA CCU-CAUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCA AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   223
MycSpe67   129  ----AUAGUA CCU-CAUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCA AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   223
CanMyc51   445  ----AUAGUA CCU-CAUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCA AGCAUUAUUC GGAUUUAUUG GGCGUAAAGC   539
CanMyc44   384  -----UGGUA CCC-UCUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCG AGCGUUAUUC GGAUUUAUUG GGCGUAAAGC   477
HmbMuris   429  -----UGGUA CCC-AGUGAA UAAGUGACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC AUAGGUCGCG AGCGUUAUUC GGAUUUAUUG GGCGUAAAGC   522
MycPneu5   475  UUUGACUGUA CCAUUUUGAA UAAGUGACGA CUAACUAUGU GCCAGCAGUC GCGGUAAUAC AUAGGUCGCA AGCGUUAUCC GGAUUUAUUG GGCGUAAAGC   574

                601        611        621        631        641        651        661        671        681        691       700
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis   562  AAGCGUAGGC GGGGA-GGCU GAUCCAUUGU UAAAGGCAUU UGCUCAACAA AUGUGUGCGA UGGAGAUCUC CUCCCUAGAG UUAAUCAGGG GGUACUGGAA   660
MycWenyo   534  AAGCGUAGGC GGGGA-GGUU GAUCCAUUGU UAAAAGCAUU UGCUUAACAA AUGUGUGCAU UGGAGAUCGC CUCCCUAGAG UUAAUCAGGG GGUACUGGAA   632
MycWeny8   269  AAGCGUAGGC GGGGA-GGUU GAUCCAUUGU UAAAAGCAUU UGCUUAACAA AUGUGUGCAU UGGAGAUCGC CUCCCUAGAG UUAAUCAGGG GGUACUGGAA   367
MycWeny9   246  AAGCGUAGGC GGGGA-GGUU GAUCCAUUGU UAAAAGCAUU UGCUUAACAA AUGUGUGCAU UGGAGAUCGC CUCCCUAGAG UUAAUCAGGG GGUACUGGAA   344
EperSuis   516  AAGCGUAGGC UGAAG-UGUG UAUCCAUUGU UAAAAGUACU UGCUUAACAA GUGUUCGCGG UGGAGAUUAC ACUUCUAGAA UUAGUUAGAG GGCACUGGAA   614
MycEryth   561  AAGCGUAGGC GGACA-GUCU GAUCCAUUGU UAAAUACAUU UGCUCAACAA AUGUAUGCAG UGGAGAUCUC CUGUCUAGAA UUAAUUAGGG GAAACUGGAA   659
HmbFeli3   538  AAGCGCAGGC GGAUGUUGUA AGUUCUGUGU UAAAUGCAGC UACUCAAUAG UUGUAUGCAC CGAAUACUAC AUGUCUAGAU UGUGGUAGGG AGUUUCGGAA   637
HmbCanis   524  AAGCGCAGGC GGAUG-UGUA AGUUCUGUGU UAAAUGCAGC UACUCAAUAG UUGUAUGCAC CGAAUACUAC AUGUCUAGAU UGUGGUAGGG AGUUUCGGAA   622
MycSpe66   224  AAGCGCAGGC GGAUG-UGUA AGUUCUGUGU UAAAAGUAGC UACUUAAUAG UUGUUUGCAC CGAAUACUAC AUGUCUAGAA UGUGGUAGGA AGUUUUGGAA   322
MycSpe68   224  AAGCGCAGGC GGAUG-UGUA AGUUCUGUGU UAAAAGUAGC UACUUAAUAG UUGUUUGCAC CGAAUACUAC AUGUCUAGAA UGUGGUAGGA AGUUUUGGAA   322
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MycSpe67   224  AAGCGCAGGC GGAUG-UGUA AGUUCUGUGU UAAAAGUAGC UACUUAAUAG UUGUUUGCAC CGAAUACUAC AUGUCUAGAA UGUGGUAGGA AGUUUUGGAA   322
CanMyc51   540  AAGCGCAGGC GGAUG-UGUA AGUUCUGUGU UAAAAGUAGC UACUUAAUAG UUGUUUGCAC CGAAUACUAC AUGUCUAGAA UGUGGUAGGA AGUUUUGGAA   638
CanMyc44   478  AAGCGCAGGC GGAUG-AAUA AGUUCUGCAU UAAAAGCAGC UGCUUAACAG UUGUUUGUGC CGAAUACUAU UCAUCUAGAA UGUGGUAGGA AGUUUUGGAA   576
HmbMuris   523  GAGCGCAGGC GGAUU-GGUA AGUUCUGUGU UAAAUGCAGC CGCUCAACGG UUGUAUGCGC AGAAUACUGC CUUUCUAGAA UACGGUAGAA AGUUUUGGAA   621
MycPneu5   575  AAGCGCAGGC GGAUU-GAAA AGUCUGGUGU UAAAGGCAGC UGCUUAACAG UUGUAUGCAU UGGAAACUAU UAAUCUAGAG UGUGGUAGGG AGUUUUGGAA   673

                      701        711        721        731        741        751        761        771        781        791       800
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   661  UUCAAUGUGU AGCGGUGGAA UGCGUAGAUA UAUUGAGGAA CACCAGAGGC UAAGGCGAGU ACCUGGGAUA UA-ACUGACG CUGAGGCUUG AAAGCGUGGG   759
MycWenyo   633  UUCAAUGUGU AGCGGUGGAA UGCGUAGAUA UAUUGAGGAA CACCAGAGGC UAAGGCGAGU GCCUAGGAUA UA-ACUGACG CUGAGGCUUG AAAGCGUGGG   731
MycWeny8   368  UUCAAUGUGU AGCGGUGGAA UGCGUAGAUA UAUUGAGGAA CACCAGAGGC UAAGGCGAGU ACCUAGGAUA UA-ACUGACG CUGAGGCUUG AAAGCGUGGG   466
MycWeny9   345  UUCAAUGUGU AGCGGUGGAA UGCGUAGAUA UAUCGAGGAA CACCAGAGGC UAAGGCGAGU GCCUAGGAUA UA-ACUGACG CUGAGGCUUG AAAGCGUGGG   443
EperSuis   615  UUCAAUGUGU AGUGGUGGAA UACGUAGAUA UAUUGAGGAA CACCAGAGGC UAAGGCGAGU GCCUGGGACA UA-AUUGACG CUGAGGCUUG AAAGCGUGGG   713
MycEryth   660  UUCAAUGUGU AGUGGUGGAA UACGUAGAUA UAUUGAGGAA CACCAAAGGC GAAAGCAAGU UUCUAGAAUA UA-AUUGACG CUGAGGCUUG AAAGCGUGGG   758
HmbFeli3   638  UUAAGCAUGG AGCGGUGGAA UGUGUAGAUA UGCUUAAGAA CACCAGAGGC GAAGGCGGAA ACUUAGGCC- AUAAAUGACG UUUAGGCUUG AAAGUGUGGG   736
HmbCanis   623  UUAAGCAUGG AGCGGUGGAA UGUGUAGAUA UGCUUAAGAA CACCAGAGGC GAAGGCGGAA ACUUAGGCC- AUAAAUGACG CUUAGGCUUG AAAGUGUGGG   721
MycSpe66   323  UUAAACAUGG AGCGGUGGAA UGUGUAGAUA UGUUUAAGAA CACCGGAGGC GAAGGCGAAA ACUUAGGCC- AUAAUUGACG CUUAGGCUUG AAAGUGUGGG   421
MycSpe68   323  UUAAACAUGG AGCGGUGGAA UGUGUAGAUA UGUUUAAGAA CACCGGAGGC GAAGGCGAAA ACUUAGGCC- AUAAUUGACG CUUAGGCUUG AAAGUGUGGG   421
MycSpe67   323  UUAAACAUGG AGCGGUGGAA UGUGUAGAUA UGUUUAAGAA CACCGGAGGC GAAGGCGAAA ACUUAGGCC- AUAAUUGACG CUUAGGCUUG AAAGUGUGGG   421
CanMyc51   639  UUAAACAUGG AGCGGUGGAA UGUGUAGAUA UGUUUAAGAA CACCGGAGGC GAAGGCGAAA ACUUAGGCC- AUAAUUGACG CUUAGGCUUG AAAGUGUGGG   737
CanMyc44   577  UUAAAUAUGG AGCGGUGGAA UGUGUAGAUA UAUUUAAGAA CACCAGAGGC GAAGGCGAAA ACUUAGGCCA UU-AUUGACG CUUAGGCUUG AAAGUGUGGG   675
HmbMuris   622  UUGAAUGUGG AGCGGUGGAA UGUGUAGAUA UAUUCAAGAA CACCAGAGGC GAAGGCGAAA ACUUAGGCC- GAUAUUGACG CUUAGGCUCG AAAGUGUGGG   720
MycPneu5   674  UUUCAUGUGG AGCGGUGAAA UGCGUAGAUA UAUGAAGGAA CACCAGUGGC GAAGGCGAAA ACUUAGGCC- AUUACUGACG CUUAGGCUUG AAAGUGUGGG   772

                      801        811        821        831        841        851        861        871        881        891       900
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   760  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGAGC AUUAGGUAUU UGAU-GUUAG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   856
MycWenyo   732  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGAGC AUUGGGCAUU UGAC-AUCUG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   828
MycWeny8   467  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGAGC AUUGGGCAUU UGAC-AUCUG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   563
MycWeny9   444  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGAGC AUUGGGCAUU UGAC-AUCUG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   540
EperSuis   714  UAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUAGUCAUU UGAA-UUUAA -GUUGAGUGA UGUAGCUAAC GCGUUAAAUA   810
MycEryth   759  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACGCCGUA AACGAUGGGU AUUUGGCAUU UGGG-CUGGA -CUUGAGUGC CGGAGCUAAC GCAUUAAAUG   855
HmbFeli3   737  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGCUUUA- GCUUUAGUGU UGUAGCUUAC GCGUUAAAUA   834
HmbCanis   722  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGCUUUA- GCUUUAGUGU UGUAGCUUAC GCGUUAAAUA   819
MycSpe66   422  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUAUU GGGGUUUGA- GCCUCAGUGC UGUAGCUAAC GUGUUAAAUA   519
MycSpe68   422  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUAUU GGGGUUUGA- GCCUCAGUGC UGUAGCUAAC GUGUUAAAUA   519
MycSpe67   422  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUGGGUAUU GGGGUUUGA- GCCUCAGUGC UGUAGCUAAC GUGUUAAAUA   519
CanMyc51   738  GAGCAAGUGG GAUUAGAUAC CCCAGUAGUU CCACACCGUA AACGAUGGGU AUUAGGUAUU GGGGUUUGA- GCCUCAGUGC UGUAGCUAAC GUGUUAAAUA   836
CanMyc44   676  UAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGAUGUC GGGAUUUGU- GUUUCGGCGU UGUAGCUUAC GUGUUAAAUA   773
HmbMuris   721  GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGAU AUUAGAUGUU GGGACUUGA- GUCUCAGCGU UGUAGCUUAC GUGUUAAAUA   818
MycPneu5   773  GAGCAAAUAG GAUUAGAUAC CCUAGUAG-U CCACACCGUA AACGAUAGAU ACUAGCUGUC GGGGCGAUC- CCCUCGGUAG UGAAGUUAAC ACAUUAAGUA   870

                      901        911        921        931        941        951        961        971        981        991      1000
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   857  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   955
MycWenyo   829  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   927
MycWeny8   564  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   662
MycWeny9   541  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   639
EperSuis   811  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU CAUACACGCA   909
MycEryth   856  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGAUCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   954
HmbFeli3   835  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   933
HmbCanis   820  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   918
MycSpe66   520  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AGACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   618
MycSpe68   520  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   618
MycSpe67   520  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   618
CanMyc51   837  CCCCGCCUGG GUAGUACAUA UGCAAAUAAG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   935
CanMyc44   774  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AACAUGUUGC UUAAUUCGAU AAUACACGAA   872
HmbMuris   819  UCCCGCCUGA GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AACAUGUUGC UUAAUUCGAC AAUACACGAA   917
MycPneu5   871  UCUCGCCUGG GUAGUACAUU CGCAAGAAUG AAACUCAAAC GGAAUUGACG GGGACCCGCA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAC GGUACACGAA   970
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Alignment 16S rRNA of bovine HM

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                1001        1011        1021        1031        1041        1051        1061        1071        1081        1091      1100
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis   956  AAACCUUACC AAGGCUUGUU AUCUACUGCA AAACUAUAGA AAUAUGGUGG -AGUAU--AU CAGUAAGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1052
MycWenyo   928  AAACCUUACC AAGGCUUGUA AUCUAUUGCG AAGCUAUAGA AAUAUAGUGG -AGGUU--AU CAGUAAGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1024
MycWeny8   663  AAACCUUACC AAGGCUUGUA AUCUAUUGCG AAGCUAUAGA AAUAUAGUGG -AGGUU--AU CAGUAAGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC   759
MycWeny9   640  AAACCUUACC AAGGCUUGUA AUCUAUUGCG AAGCUAUAGA AAUAUAGUGG -AGGUU--AU CAGUAAGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC   736
EperSuis   910  AAACCUUACC AAGGCUUGCA AUCUUCUGCA AAGCUAUAGA AAUAUAGUGG -AGGCU--AU CAGAAUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1006
MycEryth   955  AAACCUUACC AAGGCUUGUA AUCUUCUGCA AAAGUGUAGA AAUACAGUGG -AGUAU--AU CAGAAUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1051
HmbFeli3   934  AAACCUUACC AAGGUUUGAC AUCCCUCGCA AAGCUAUAGA AAUAUAGUAG A-G-GUUA-U CGAGGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1030
HmbCanis   919  AAACCUUACC AAGGUUUGAC AUCCCUCGCA AAGCUAUAGA AAUAUAGUAG A-G-GUUA-U CGAGGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1015
MycSpe66   619  AAACCUUACC AAGGUUUGAC AUCCUUCGCA AAGCUAUAGA AAUAUAGUAG -AGGUU--AU CGGAGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC   715
MycSpe68   619  AAACCUUACC AAGGUUUGAC AUCCUUCGCA AAGCUAUAGA AAUAUAGUAG -AGGUU--AU CGGAGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC   715
MycSpe67   619  AAACCUUACC AAGGUUUGAC AUCCUUCGCA AAGCUAUAGA AAUAUAGUAG -AGGUU--AU CGGAGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC   715
CanMyc51   936  AAACCUUACC AAGGUUUGAC AUCCUUCGCA AAGCUAUAGA AAUAUAGUAG A-G-GUUA-U CGGAGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1032
CanMyc44   873  AAACCUUACC AAGGUUUGAC AUCCUUUGCA AAGCCAUAGA AAUAUGGUGG -AG-GUUA-U CAGAGUGACA GGUGGUGCAU GGUUGUCGUC AGCUCGUGUC   969
HmbMuris   918  AAACCUUACC AAGAUUUGAC AUCCCCUGCG AAGCUUUAGA AAUAAAGUGG -AG-GUUA-U CAGGGUGACA GGUGGUGCAU GGCUGUCGUC AGCUCGUGUC  1014
MycPneu5   971  AAACCUUACC UAGACUUGAC AUCCUUGGCA AAGUUAUGGA AACAUAAUGG -AG-GUUA-A CCGAGUGACA GGUGGUGCAU GGUUGUCGUC AGCUCGUGUC  1067

                1101        1111        1121        1131        1141        1151        1161        1171        1181        1191      1200
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis  1053  UUGAGAUGUU UGGUUAAGUC CCGUAACGAG CGCAACCCUA CUCUCUAG-U U-AAUU-AGU UCUAGAGUGA CUGA-AUCGU AAGAU-AUAG GAAGGAUGGG  1147
MycWenyo  1025  UUGAGAUGUU UGGUUAAGUC CCGUAACGAG CGCAACCCUA CUCUCUAG-U U-ACUU-AGU UCUAGAGUGA CUGA-AUCGU AAGAU-AUAG GAAGGAUGGG  1119
MycWeny8   760  UUGAGAUGUU UGGUUAAGUC CCGUAACGAG CGCAACCCUA CUCUCUAG-U U-ACUU-AGU UCUAGAGUGA CUGA-AUCGU AAGAU-AUAG GAAGGAUGGG   854
MycWeny9   737  UUGAGAUGUU UGGUUAAGUC CCGUAACGAG CGCAACCCUA CUCUCUAG-U U-ACUU-AGU UCUAGAGUGA CUGA-AUCGU AAGAU-AUAG GAAGGAUGGG   831
EperSuis  1007  UUGAGAUGUU UGGUAAAGUC CCGUAACGAG CGCAACCCUU CAUAUUAGUU G-UUU--AGU UCUAAUAUGA CUGA-AUCGU AAGAU-CUAG GAAGGAUGGG  1101
MycEryth  1052  UUGAGAUGUU UGGUUAAGUC CCGUAACGAG CGCAACCCCU CUCAUUAG-U U-AAAU-AGU UCUAAUGUGA CUGA-AUCGU AAGAU-AUAG GAAGGAUGGG  1146
HmbFeli3  1031  UUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCCA CUCUUUAG-U UA--C-UUG- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGAUGGG  1123
HmbCanis  1016  UUGAGAUGUU UGGUAAAGUC CCGCAACGAG CGCAACCCCA CUCUUUAG-U UA--C-UUG- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGAUGGG  1108
MycSpe66   716  UUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCUA CUCUUUAG-U UA--U-UUU- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGUUGGG   808
MycSpe68   716  UUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCUA CUCUUUAG-U UA--U-UUU- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGUUGGG   808
MycSpe67   716  UUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCUA CUCUUUAG-U UA--U-UUU- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGUUGGG   808
CanMyc51  1033  UUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCUA CUCUUUAG-U UA--U-UUU- UCUAAAGAGA CUGC-ACAGU AAUGU-AGAG GAAGGUUGGG  1125
CanMyc44   970  AUGAGAUGUU UGGUUAAGUC CCGCAACGAG CGCAACCCUA CUCUUUAG-U UG--A-UUG- UCUAAAGAGA CUGA-ACAGU AAUGU-AUAG GAAGGAUGGG  1062
HmbMuris  1015  AUGAGAUGUC UGGUUAAGUC CUGAAACGAG CGCAACCCUA CUCUUUAG-U UA--A-CUU- UCUAAAGAGA CUGA-ACAGU AAUGU-AUAG GAAGGAUGGG  1107
MycPneu5  1068  GUGAGAUGUU GGGUUAAGUC CCGCAACGAG CGCAACCCUU AUCGUUAG-U UA--CAUUG- UCUAGCGAGA CUGCUAAUGC AAAUUG-GAG GAAGGAAGGG  1162

                1201        1211        1221        1231        1241        1251        1261        1271        1281        1291      1300
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis  1148  GCCAAGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGUAGGU ACAAUGUGUU GCAAUCUAG- CGAUAGUGAG CUAAUCACCG  1246
MycWenyo  1120  GCCAAGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGUAGGU ACAAUGUGUU GCAAACUAG- CGAUAGUGAG CCAAUCACCU  1218
MycWeny8   855  GCCAAGUCAA GUCAUCAUGC CCCUUAUGCC CUGGGCUGCA AACGUGCUAC AAUGGUAGGU ACAAUGUGUU GCAAACUAG- CGAUAGUGAG CCGAUCACCU   953
MycWeny9   832  GCCAGGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGUAGGU ACAAUGUGUU GCAAACUAG- CGAUAGUGAG CCAAUCACCU   930
EperSuis  1102  GCCAAGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGUAGAU ACAAUGUGUU ACAAUCUAGG CGAUAGUGAG UUAAUCACCU  1201
MycEryth  1147  GCCAAGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGUAGGU ACAAUGUGUU GCAACUUAG- CGAUAAUGAG CUAAUCACCG  1245
HmbFeli3  1124  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCGAAC ACAAUGUGUU GCAAACCAG- CGAUGGUAAG CUAAUCACC-  1221
HmbCanis  1109  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCGAAC ACAAUGUGUU GCAAACCAG- CGAUGGUAAG CUAAUCACC-  1206
MycSpe66   809  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCAAAC ACAAUGUGUU GCAAAUCAG- CGAUGAUGAG CUAAUCACU-   906
MycSpe68   809  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCAAAC ACAAUGUGUU GCAAAUCAG- CGAUGAUGAG CUAAUCACU-   906
MycSpe67   809  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCAAAC ACAAUGUGUU GCAAAUCAG- CGAUGAUGAG CUAAUCACU-   906
CanMyc51  1126  AUCACGUCAA GUCAUCAUGC CCCUUAUGCC UUGGGCUGCA AACGUGCUAC AAUGGCAAAC ACAAUGUGUU GCAAAUCAG- CGAUGAUGAG CUAAUCACU-  1223
CanMyc44  1063  AUCACGUCAA AUCAUCAUGC CCCUUAUGCC UUGGGCCGCA AACGUGUUAC AAUGGUGAGU ACAAUGUGUC GCGAACCAG- CGAUGGUAAG CUAAUCAC-C  1160
HmbMuris  1108  AUCACGUCAA GUCAUCAUGC CCCUUAUAUC UUGGGCCGCA AACGUGUUAC AAUGGUGGGU ACAACGUGUC GCAAGCCAG- CGAUGGCAAG CCAAUCAC-U  1205
MycPneu5  1163  AUGACGUCAA AUCAUCAUGC CCCUUAUGUC UAGGGCUGCA AACGUGCUAC AAUGGCCAAU ACAAACAGUC GCCAGCUUG- UAAAAGUGAG CAAAUCUG-U  1260

                1301        1311        1321        1331        1341        1351        1361        1371        1381        1391      1400
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis  1247  AAA-ACCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGU UGGAAUCACU AGUAAUCCUG UGUCAGCUAU AUCAGGGUGA AUACGUUCCC  1345
MycWenyo  1219  AAA-GCCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGU UGGAAUCACU AGUAAUCCUG UGUCAGCUAU AUCAGGGUGA AUACGUUCCC  1317
MycWeny8   954  AAA-GCCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGU UGGAAUCACU AGUAAUCCUG UGUCAGCUAU AUCAGGGUGA AUACGUUCCC  1052
MycWenv9   931  AAA-GCCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGU UGGAAUCACU AGUAAUCCC. .......... .......... ..........   998
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Alignment 16S rRNA of bovine HM

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EperSuis  1202  AAA-GUCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGA UGGAAUCACU AGUAAUCCUG UGUCAGCUAU AUCAGGGUGA AUACGUUCCC  1300
MycEryth  1246  AAA-ACCUAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UAUUUGAAGA UGGAAUCACU AGUAAUCCUG UGUCAGCUAU AUCAGGGUGA AUGCGUUCCC  1344
HmbFeli3  1222  AAA-UUUCGU CUCAGUUCGG AUAGGAGGCU GCAAUUCGCC UCCUUGAAGU UGGAAUCACU AGUAAUCCCG UGUCAGCUAU AUCGGGGUGA AUCCGUUCCC  1320
HmbCanis  1207  AAA-UUUCGU CUCAGUUCGG AUAGGAGGCU GCAAUUCGCC UCCUUGAAGU UGGAAUCACU AGUAAUCCCG UGUCAGCUAU AUCGGGGUGA AUCCGUUCCC  1305
MycSpe66   907  AAA-UUUUGU CUCAGUUCGG AUAGAAGGCU GCAAUUCGCC UUCUUGAAGU UGGAAUCACU AGUAAUCCCG .......... .......... ..........   975
MycSpe68   907  AAA-UUUUGU CUCAGUUCGG AUAGAAGGCU GCAAUUCGCC UUCUUGAAGU UGGAAUCACU AGUAAUCCCG .......... .......... ..........   975
MycSpe67   907  AAA-UUUUGU CUCAGUUCGG AUAGAAGGCU GCAAUUCGCC UUCUUGAAGU UGGAAUCACU AGUAAUCCCG .......... .......... ..........   975
CanMyc51  1224  AAA-UUUUGU CUCAGUUCGG AUAGAAGGCU GCAAUUCGCC UUCUUGAAGU UGGAAUCACU AGUAAUCCCG UGUCAGCUAU AUCGGGGUGA AUGCGUUCCC  1322
CanMyc44  1161  AAA-ACUCAU CUCAGUCCGG AUAAAAGGCU GCAAUUCGCC UUUUUGAAGU UGGAAUCACU AGUAAUCCCG UGUCAGCUAU AUCGGGGUGA AUACGUUCCC  1259
HmbMuris  1206  AAAAGCCCAU CCCAGUCCGG AUAAAAGGCU GCAAUUCGCC UUUUUGAAGU UGGAAUCACU AGUAAUCCCG UGUCAGCCAU AUCGGGGUGA AUACGUUCCC  1305
MycPneu5  1261  AAA-GUUGGU CUCAGUUCGG AUUGAGGGCU GCAAUUCGUC CUCAUGAAGU CGGAAUCACU AGUAAUCGCG AAUCAGCUAU GUCGCGGUGA AUACGUUCUC  1359

                1401       1411       1421       1431       1441       1451       1461       1471       1481       1491      1500
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis  1346  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAAAGAAAGU AUCAGUCAAA ACCGCA-U-- -----UCAA- -----UUGUG UCUAGAUUGG UAAUUUUGAU  1431
MycWenyo  1318  AGGUCUUGUA CACACCGCCC GUCAAACUAC GAAAGAAAGU ACUAGUCAAA ACCACA-U-- -----UUAA- -----UUGUG UCUAGAUUGG UAAUUU....  1399
MycWeny8  1053  AGGUCUUGUA CACACCGCCC G......... .......... .......... .......... .......... .......... .......... ..........  1073
MycWeny9   999  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   998
EperSuis  1301  AGGUGUUGUA CACACCGCCC GUCAAACUAC GAAAGAAAGU ACUAAUUAAA ACCGUA-U-- -----UUAA- -----UUACG UCUAGAUU.. ..........  1374
MycEryth  1345  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAAAGAAAGU ACUAGUUAAA ACCGCA-U-- -----UUAA- -----UUGCG UCUAGAUUGG UAAUUUUAAU  1430
HmbFeli3  1321  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAGAGGAGUG GGCAUUUAAA AAUACA-U.. .......... .......... .......... ..........  1377
HmbCanis  1306  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAGAGGAGUG GGCAUUUAAA AAUACA-U-- -----UUAU- -----UUGUA UCUAGAGUGA ACAU-CUGAU  1390
MycSpe66   976  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   975
MycSpe68   976  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   975
MycSpe67   976  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   975
CanMyc51  1323  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAGAGGAGUG GGCAUUUAAA AAUGUA-U-- -----UCAU- -----UUGCA UCUAGAGUGA ACAUUCUGAG  1408
CanMyc44  1260  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAGAGGAAGG GGCAUUUGAA AACACA-U-- -----UCAA- -----UUGUG UCAAGAAUGA AACUUC....  1341
HmbMuris  1306  AGGUCUUGUA CACACCGCCC GUCAAACUAU GAGAGGGAGA GGCAUUCGAA AACGCA-U-- -----UCAU- -----UUGCG UCUAGAAUGA AUUUUCCGAU  1391
MycPneu5  1360  GGGUCUUGUA CACACCGCCC GUCAAACUAU GAAAGCUGGU AAUAUUUAAA AACGUGUUGC UAACCAUUAG GAAGCGCAUG UCAAGGAUAG CACCGGUGAU  1459

                1501       1511       1521       1531       1541       1551
                |          |          |          |          |          |
MycoOvis  1432  UGGAGUUAAG UCGUAACAAG GUAACCG... .......... .......... ...                                                  1458
MycWenyo  1400  .......... .......... .......... .......... .......... ...                                                  1399
MycWeny8  1074  .......... .......... .......... .......... .......... ...                                                  1073
MycWeny9   999  .......... .......... .......... .......... .......... ...                                                   998
EperSuis  1375  .......... .......... .......... .......... .......... ...                                                  1374
MycEryth  1431  UGGAGUUAAG UCGUAACAAG GUA....... .......... .......... ...                                                  1453
HmbFeli3  1378  .......... .......... .......... .......... .......... ...                                                  1377
HmbCanis  1391  UGGAGUU... .......... .......... .......... .......... ...                                                  1397
MycSpe66   976  .......... .......... .......... .......... .......... ...                                                   975
MycSpe68   976  .......... .......... .......... .......... .......... ...                                                   975
MycSpe67   976  .......... .......... .......... .......... .......... ...                                                   975
CanMyc51  1409  UGGAGUUAAG UCGUAACAAG GUAGCCG... .......... .......... ...                                                  1435
CanMyc44  1342  .......... .......... .......... .......... .......... ...                                                  1341
HmbMuris  1392  UGGAGUUAAG .......... .......... .......... .......... ...                                                  1401
MycPneu5  1460  UGGAGUUAAG UCGUAACAAG GUACCCCUAC GAGAACGUGG GGGUGGAUCA CCU                                                   1512
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Alignment 16S rRNA of equine HM

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                1          11         21         31         41         51         61         71         81         91        100
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe18      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe16      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe17      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
CanMyc51      1 AGAGUUUGAU CCUGGCUCAG AAUUAAUGCU GAUGGUAUGC CUAAUACAUG CAAGUCGAAC GGACUUUGGU UUCGGCCAAA GUUAGUGGCG AACGGGUGAG  100

                101        111        121        131        141        151        161        171        181        191       200
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe18      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe16      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe17      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
CanMyc51    101 UAAUACAUAU CUAACAUGCC CCUCUGUAGG GAAUAGCCAC UUGAAAAGGU AAUUAAUACC CUAUAGGUAA CUUUCUCACA AGAGUUAGUU AUUAAAGCUU  200

                201        211        221        231        241        251        261        271        281        291       300
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe18      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe16      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe17      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
CanMyc51    201 UAUGCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUUGUUG GCGGGGUAAA AGCCCACCAA GGCUAUGAUA GAUAGCUGGU CAUAGAGGAU GAACAGCCAC  300

                301        311        321        331        341        351        361        371        381        391       400
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe18      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe16      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe17      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
CanMyc51    301 AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG UCUGAUGGAG CAAUACCAUG UGAACGAUGA  400

                401        411        421        431        441        451        461        471        481        491       500
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe18      1 .......... .......... .......... .......... .......... .......... .......... .......... ....GCUGCU GCGGUAAUAC   16
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 0
MycSpe16      1 .......... .......... .......... .......... .......... .......... AAGUGGACAG C-AACUAUGU GCCAGCAGCU GCGGUAAUAC   39
MycSpe17      1 .......... .......... .......... .......... .......... .......... .AGUG-ACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC   38
CanMyc51    401 AGGUCUUUUU GAUUGUAAAG UUCUUUUAUG AGGGAUAACA ACUGAUAGUA CCUCAUGAAU AAGUG-ACAG CAAACUAUGU GCCAGCAGCU GCGGUAAUAC  499

                501        511        521        531        541        551        561        571        581        591       600
                |          |          |          |          |          |          |          |          |          |         |
MycSp111      1 .......... .......... .......... .......... .......... .......... .......... .......... .......ATA GTTGTTTGCA   13
MycSpe18     17 AAUAGGUCGC AAGCAUUUUC CGGGAUUUAU UGGGGCGUAAA GCAAGCGCAG GCGGAUGUGU AAGUUCUGUG UUAAAAGUAG CUACUUAAUA GUUGUUUGCA  116
MycSp112      1 .......... .......... .......... .......... .......... .......... .......... .......... .......ATA GTTGTTTGCA   13
MycSp110      1 .......... .......... .......... .......... .......... .......... .......... .......... .......ATA GTTGTTTGCA   13
MycSpe16     40 A-UAGGUCGC AAGCAUUAUU C-GGAUUUAU UGGGCGUAAA GCAAGCGCAG GCGGAUGUGU AAGUUCUGUG UUAAAGGUAG CUACUUAAUA GUUGUUUGCA  137
MycSpe17     39 A-UAGGUCGC AAGCAUUAUU C-GGA-UUAU UGGGCGUAAA GCAAGCGCAG GCGGAUGUGU AAGUUCUGUG UUAAAAGUAG CUACUUAAUA GU-GUUUGCA  134
CanMyc51    500 A-UAGGUCGC AAGCAUUAUU C-GGAUUUAU UGGGCGUAAA GCAAGCGCAG GCGGAUGUGU AAGUUCUGUG UUAAAAGUAG CUACUUAAUA GUUGUUUGCA  597
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Alignment 16S rRNA of equine HM

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                601        611        621        631        641        651        661        671        681        691       700
                |          |          |          |          |          |          |          |          |          |         |
MycSp111     14 CCGAATACTA CATGTCTAGA ATGTGGTAGG AAGTTTTGGA ATTAAACACG GAGCGGTGGA GTGTGTAGAT ATGTTTAAGA ACACCGGAGG CGA.......   106
MycSpe18    117 CCGAAUACUA CAUGUCUAGA AUGUGGUAGG AAGUUUUGGA AUUAAACACG GAGCGGUGGA GUGUGUAGAU AUGUUUAAGA ACACCGGAGG CGAAGGCGAA   216
MycSp112     14 CCGAATACTA CATGTCTAGA ATGTGGTAGG AAGTTTTGGA ATTAAACACG GAGCGGTGGA GTGTGTAGAT ATGTTTAAGA ACACCGGAGG CGAATTA...   110
MycSp110     14 CCGAATACTA CATGTCTAGA ATGTGGTAGG AAGTTTTGGA ATTAAACACG GAGCGGTGGA GTGTGTAGAT ATGTTTAAGA ACACCGGAGG CAAA......   107
MycSpe16    138 CCGAAUACUA CAUGUCUAGA AUGUGGUAGG AAGUUUUGGA AUUAAACAUG GAGCGGUGGA AUGUGUAGAU AUGUUUAAGA ACACCGGAGG CGAAGGCGAA   237
MycSpe17    135 CCGAAUACUA CAUGUCUAGA AUGUGGUAGG AAGUUUUGGA AUUAAACAUG GAGCGGUGGA AUGUGUAGAU AUGUUUAAGA ACACCGGAGG CGAAGGCGAA   234
CanMyc51    598 CCGAAUACUA CAUGUCUAGA AUGUGGUAGG AAGUUUUGGA AUUAAACAUG GAGCGGUGGA AUGUGUAGAU AUGUUUAAGA ACACCGGAGG CGAAGGCGAA   697

                701        711        721        731        741        751        761        771        781        791       800
                |          |          |          |          |          |          |          |          |          |         |
MycSp111    107 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18    217 AACUUAGGCC AUAAUUGACG CUUAGGCUUG AAAGUGUGGG GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUAUU   315
MycSp112    111 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110    108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16    238 AACUUAGGCC AUAAUUGACG CUUAGGCUUG AAAGUGUGGG GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUAUU   336
MycSpe17    235 AACUUAGGCC AUAAUUGACG CUUAGGCUUG AAAGUGUGGG GAGCAAAUGG GAUUAGAUAC CCCAGUAG-U CCACACCGUA AACGAUGGGU AUUAGGUAUU   333
CanMyc51    698 AACUUAGGCC AUAAUUGACG CUUAGGCUUG AAAGUGUGGG GAGCAAGUGG GAUUAGAUAC CCCAGUAGUU CCACACCGUA AACGAUGGGU AUUAGGUAUU   797

                801        811        821        831        841        851        861        871        881        891       900
                |          |          |          |          |          |          |          |          |          |         |
MycSp111    107 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18    316 GGGGUUUGAG CCUCAGUGCU GUAGCUAACG UGUUAAAUAC CCCGCCUGGG UAGUACAUAU GCAAAUAUGA AACUCAAAGG AAUUGACGGG GACCUGAACA   415
MycSp112    111 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110    108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16    337 GGGGUUUGAG CCUCAGUGCU GUAGCUAACG UGUUAAGUAC CCCGCCUGGG UAGUACAUAU GCAAAUAUGA AACUCAAAGG AAUUGACGGG GACCUGAACA   436
MycSpe17    334 GGGGUUUGAG CCUCAGUGCU GUAGCUAACG UGUUAAAUAC CCCGCCUGGG UAGUACAUAU GCAAAUAUGA AACUCAAAGG AAUUGACGGG GACCUGAACA   433
CanMyc51    798 GGGGUUUGAG CCUCAGUGCU GUAGCUAACG UGUUAAAUAC CCCGCCUGGG UAGUACAUAU GCAAAUAAGA AACUCAAAGG AAUUGACGGG GACCUGAACA   897

                901        911        921        931        941        951        961        971        981        991      1000
                |          |          |          |          |          |          |          |          |          |         |
MycSp111    107 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18    416 AGUGGUGGAG CAUGUUGCUU AAUUCGAUAA UACACGAAAA ACCUUACCAA GGUUUGACAU CCUUCGCAAA GCUAUAGAAA UAUAGUAG-A GGUU--AUCG   512
MycSp112    111 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110    108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16    437 AGUGGUGGAG CAUGUUGCUU AAUUCGAUAA UACACGAAAA ACCUUACCAA GGUUUGACAU CCUUCGCAAA GCUAUAGAAA UAUAGUAG-A GGUU--AUCG   533
MycSpe17    434 AGUGGUGGAG CAUGUUGCUU AAUUCGAUAA UACACGAAAA ACCUUACCAA GGUUUGACAU CCUUCGCAAA GCUAUAGAAA UAUAGUAG-A GGUU--AUCG   530
CanMyc51    898 AGUGGUGGAG CAUGUUGCUU AAUUCGAUAA UACACGAAAA ACCUUACCAA GGUUUGACAU CCUUCGCAAA GCUAUAGAAA UAUAGUAGA- G-GUUA-UCG   994

                1001       1011       1021       1031       1041       1051       1061       1071       1081       1091     1100
                |          |          |          |          |          |          |          |          |          |         |
MycSp111    107 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18    513 GAGUGACAGG UGGUGCAUGG CUGUCGUCAG CUCGUGUCUU GAGAUGUUUG GUUAAGUCCC GCAACGAGCG CAACCCUACU CUUUAGUUAU UUUUCUAAAG   612
MycSp112    111 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110    108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16    534 GAGUGACAGG UGGUGCAUGG CUGUCGUCAG CUCGUGUCUU GAGAUGUUUG GUUAAGUCCC GCAACGAGCG CAACCCUACU CUUUAGUUAU UUUUCUAAAG   633
MycSpe17    531 GAGUGACAGG UGGUGCAUGG CUGUCGUCAG CUCGUGUCUU GAGAUGUUUG GUUAAGUCCC GCAACGAGCG CAACCCUACU CUUUAGUUAU UUUUCUAAAG   630
CanMyc51    995 GAGUGACAGG UGGUGCAUGG CUGUCGUCAG CUCGUGUCUU GAGAUGUUUG GUUAAGUCCC GCAACGAGCG CAACCCUACU CUUUAGUUAU UUUUCUAAAG  1094

                1101       1111       1121       1131       1141       1151       1161       1171       1181       1191     1200
                |          |          |          |          |          |          |          |          |          |         |
MycSp111    107 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18    613 AGACUGCACA GUAAUGUAGA GGAAGGUUGG GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCAAC CACAAUGUGU   712
MycSp112    111 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110    108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16    634 AGACUGCACA GUAAUGUAGA GGAAGGUUGG GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCAAA CACAAUGUGU   733
MycSpe17    631 AGACUGCACA GUAAUGUAGA GGAAGGUUGG GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCAAA CACAAUGUGU   730
CanMyc51   1095 AGACUGCACA GUAAUGUAGA GGAAGGUUGG GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCAAA CACAAUGUGU  1194
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Alignment 16S rRNA of equine HM

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                1201       1211       1221       1231       1241       1251       1261       1271       1281       1291      1300
                |          |          |          |          |          |          |          |          |          |         |
MycSp111   107  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18   713  UGCAAAUCAG CGAUGAUGAG CUAAUCACUA UAUUUUGUCU CAGUUCGGAU AGAAGGCUGC AAUUCGCCUU CUUGAAGUUG GAAUCACUAG UAAUCCCGU.   811
MycSp112   111  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110   108  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16   734  UGCAAAUCAG CGAUGAUGAG CUAAUCACUA AAUUUUGUCU CAGUUCGGAU AGAAGGCUGC AAUUCGCCUU CUUGAAGUUG GAAUCACUAG UAAUCCCG..   831
MycSpe17   731  UGCAAAUCAG CGAUGAUGAG CUAAUCACUA AAUUUUGUCU CAGUUCGGAU AGAAGGCUGC AAUUCGCCUU CUUGAAGUUG GAAUCACUAG UAAUCCCGU.   829
CanMyc51  1195  UGCAAAUCAG CGAUGAUGAG CUAAUCACUA AAUUUUGUCU CAGUUCGGAU AGAAGGCUGC AAUUCGCCUU CUUGAAGUUG GAAUCACUAG UAAUCCCGUG  1294

                1301       1311       1321       1331       1341       1351       1361       1371       1381       1391      1400
                |          |          |          |          |          |          |          |          |          |         |
MycSp111   107  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   106
MycSpe18   812  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   811
MycSp112   111  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   110
MycSp110   108  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   107
MycSpe16   832  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   831
MycSpe17   830  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   829
CanMyc51  1295  UCAGCUAUAU CGGGGUGAAU GCGUUCCCAG GUCUUGUACA CACCGCCCGU CAAACUAUGA GAGGAGUGGG CAUUUAAAAA UGUAUUCAUU UGCAUCUAGA  1394

                1401       1411       1421       1431       1441
                |          |          |          |          |
MycSp111   107  .......... .......... .......... .......... .                                                              106
MycSpe18   812  .......... .......... .......... .......... .                                                              811
MycSp112   111  .......... .......... .......... .......... .                                                              110
MycSp110   108  .......... .......... .......... .......... .                                                              107
MycSpe16   832  .......... .......... .......... .......... .                                                              831
MycSpe17   830  .......... .......... .......... .......... .                                                              829
CanMyc51  1395  GUGAACAUUC UGAGUGGAGU UAAGUCGUAA CAAGGUAGCC G                                                             1435
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Alignment 16S rRNA of *Mycoplasma suis*

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                1          11         21         31         41         51         61         71         81         91        100
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis        1 ........AG AGUUUGAUCC UGGCUCAGGA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA GUAG--AACU UGUU--CUGC UAGUGGCAAA    88
MycWenyo        1 .......... .......... .......... .UAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA GUGG--AACU UGUU--CUGC UAGUGGCGAA    65
MycoSu10        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu21        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu22        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu16        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu17        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu11        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu19        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu20        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu18        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui6        1 ....CTTCAG AGTTTGATGC TGGCTCAGGA TTAATGCTGG TGGTATGCAT AACACATGCA AGTCGAACGA AAAAGGCCCT CGGGTCTTTT TAGTGGCAAA    96
MycoSui7        1 ....CTTCAG AGTTTGATGC TGGCTCAGGA TTAATGCTGG TGGTATGCAT AACACATGCA AGTCGAACGA AAAAGGCCCT CGGGTCTTTT TAGTGGCAAA    96
EperSui2        1 .......... .......... ........GA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA AAAAGGCCCU CGGGUCUUUU UAGUGGCAAA    72
EperSuis        1 .......... .......... .......... .......... .......... .......... .........A AAAAGGCCCU CGGGUCUUUU UAGUGGCAAA    31
MycoSu13        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui9        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu12        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu14        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu15        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSuis        1 ........AG AGUUUGAUCC UGGCUCAGGA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA AAAAGGUCUU CGAGCCUUUU UAGUGGCAAA    92
MycoSui2        1 ........AG AGUUUGAUCC UGGCUCAGGA UUAAUGCUGG UGGUAUGCAU AACACAUGCA AGUCGAACGA AAAAGGCCCU CGGUCCUUUU UAGUGGCAAA    92
MycoSui3        1 ........AG AGTTTGATCC TGGCTCAGGA TTAATGCTGG TGGTATGCAT AACACATGCA AGTCGAACGA AAAAGGCCCT CGGTCCTTTT TAGTGGCAAA    92
MycoSui4        1 ........AG AGTTTGATCC TGGCTCAGGA TTAATGCTGG TGGTATGCAT AACACATGCA AGTCGAACGA AAAAGGCCCT CGGGCCTTTT TAGTGGCAAA    92
HmbFeli3        1 ........AG A-UUUGAUCC UGGCUCAGAA UUAAUGCUGA UGGUAUGCCU AAUACAUGCA AGUCGAACGG AUCUUGGUUU CGGCCAAGAU UAGUGGCAAA    91
HmbCanis        1 .......... .......... ...CUCAGAA UUAAUGCUGA UGGUAUGCCU AAUACAUGCA AGUCGAACGG ACCUUGGUUU CGGCCAAGGU UAGUGGCAAA    77
MycCocco        1 ........AG AGUUUGAUCC UGGCUCAGAA UUAACGCUGG UGGCAUGCCU AAUACAUGCA AGUCGAACGA AUGUGCCCGC AAGGGUACGU UAGUGGCGAA    92
HmbMuris        1 .......... .......... ...CUCAGAA UUAACGCUGA UGGCAUACCU AAUACAUGCA AGUCGAGCGG ACCU-CUAGC AAUAG-AGGU UAGCGGCGAA    75
MycPneu5        1 UUUUUCUGAG AGUUUGAUCC UGGCUCAGGA UUAACGCUGG CGGCAUGCCU AAUACAUGCA AGUCGAUCGA AAGU---AGU AAU---ACUU UAGAGGCGAA    94

                101        111        121        131        141        151        161        171        181        191       200
                |          |          |          |          |          |          |          |          |          |          |
MycoOvis       89 CGGGGCGAGUA AUGCAUAUUU AACUUACUUU CGCGAGGAGG AUAGCAGCCC GAAAGGGCUA UUAAUACUAC AUAG-GUUUA UGG------- -AC----UU-   174
MycWenyo       66 CGGGGCGAGUA AUACAUAUUU AACUUACUUU UACGAGGAGG AUAGCAGCUC GAAAGGGCUA UUAAUACUCC AUAG-GUUUA --------UA AAC-------   149
MycoSu10        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu21        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu22        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu16        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu17        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu11        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu19        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu20        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu18        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui6       97 CGGGCGAGTA ACGCATACTT AACTTACTTA TCTGAGGAAA ATAGCAGCTC GAAAGAGCTA TTAATAATCC ATAG-GTTTA GGC-TAGAGG -AA-CTAGC-   191
MycoSui7       97 CGGGCGAGTA ACGCATACTT AACTTACTTA TCTGAGGAAA ATAGCAGCTC GAAAGAGCTA TTAATAATCC ATAG-GTTTA GGC-TAGAGG -AA-CTAGC-   191
EperSui2       73 CGGGCGAGUA ACGCAUACUU AACUUACUUA UCUGAGGAAA AUAGCAGCUC GAAAGAGCUA UUAAUAAUCC AUAG-GUUUA GGC-UAGAGG -AA-CUAGC-   167
EperSuis       32 CGGGCGAGUA ACGCAUACUU AACUUACUUA UCUGAGGAAA AUAGCAGCUC GAAAGAGCUA UUAAUAAUCC AUAG-GUUUA GGC-UAGAGG -AA-CUAGC-   126
MycoSu13        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui9        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu12        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu14        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu15        1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSuis       93 CGGGCGAGUA ACACAUAUUU AACUUGCUCA UCCGAGGAGA AUAGCAGCCC GAAAGGGCUA UUAAUACGCC AUAG-UUUUA AAU-UAGUG- -AA-UUAAU-   186
MycoSui2       93 CGGGCGAGUA ACUCAUACUU AACUAGCUUA UCUGAGGAAA AUAGCAGCUC GAAAGAGCGA UUAAUAAUCC AUAG-G.UUA GGC-UAGUGG -AA-CUAGC-   186
MycoSui3       93 CGGGCGAGTA ACTCATAGTT AACTAGCTTA TCTGAGGAAA ATAGCAGCTC GAAAGAGCGA TTAATAACCC ATAG-GTTAC GCT-CA-TGG -AA-CTAGC-   186
MycoSui4       93 CGGGCGAGTA ACTGATACAT AACTACCTTA TCAGAGGACA ATAGCAGCTC GAAAGAGACA TTAATAAACC ATAG-GTTTA GGA-TAG-GG -AA-CTACC-   186
HmbFeli3       92 CGGGUGAGUA AUACAUAUCU AACAUGCCCC UCUGUGGGGG AUAGCCGCUU GAAAAAGCGA UUAAUACCCC AUAG-GAAGC UUU-A-UC-- -UAUGAUUU-   184
HmbCanis       78 CGGGUGAGUA AUACAUAUCU AACAUGCCCC UCUGUGGGGG AUAGCCACUU GAAAAAGUGA UUAAUACCCC AUAG-GAAGC UUU-A-UC-- -CAUGAUUU-   170
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Alignment 16S rRNA of *Mycoplasma suis*

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MycCocco     93  CGGGUGAGUA AUACAUAUUU AACAUACCCC UUAGAGGGAA AUAGCCGUCU GAAAAGACGA UUAAUGUCCC AUAG-GAACC CCC-U-CA-- -C-AGGAGGG   185
HmbMuris     76  CGGGUGAGUA AUGAAUACUU AACAUACCUC CAUGAAGGAA AUAGCUAUUC GAAAGAGUAA UUAAUGUCCU AUAG-GAGCC UUCCU-CA-- -C-AUGAGGU   169
MycPneu5     95  CGGGUGAGUA ACACGUAUCC AAUCUACCUU AUAAUGGGGG AUAACUAGUU GAAAGACUAG CUAAUACCGC AUAAGAACUU UGG-U-UC-G -CAUGAAUC-   189

                 201        211        221        231        241        251        261        271        281        291       300
                 |          |          |          |          |          |          |          |          |          |         |
MycoOvis    175  GUAAAUUAAA GGAGGCGCCC -UC-GGGAG- CC-UCGCGCG GAAAAGGGAA UAUGUCCUAU UAGGUAGUUG GCGGGGUAAA GGCCCACCAA GCCAAUGAUG   270
MycWenyo    150  -UAAAUUAAA -GAGGCUCCU -C--UGGGG- CC-UUGCGUA AAACUAGGAA UAUGUCCUAU UAGGUAGUUG GCGGGGUAAA GGCCCACCAA GCCAAUGAUG   242
MycoSu10      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu21      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu22      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu16      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu17      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu11      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu19      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu20      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu18      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui6    192  TTAAATTAAA GGAGGCTGCC GCAAGGTGG- CC-TTGCGGG TAAATAGGAG TATGTCCTAT TAGATAGTTG GAGAGGTAAG GGCTCACCAA GTCGATGATG   289
MycoSui7    192  TTAAATTAAA GGAGGCTGCC GCAAGGTGG- CC-TTGCGGG TAAATAGGAG TATGTCCTAT TAGATAGTTG GAGAGGTAAG GGCTCACCAA GTCGATGATG   289
EperSui2    168  UUAAAUUAAA GGAGGCUGCC GCAAGGUGG- CC-UUGCGGG UAAAUAGGAG UAUGUCCUAU UAGAUAGUUG GAGAGGUAAA GGCUCACCAA GUCGAUGAUG   265
EperSuis    127  UUAAAUUAAA GGAGGCUGCC GCAAGGUGG- CC-UUGCGGG UAAAUAGGAG UAUGUCCUAU UAGAUAGUUG GAGAGGUAAA GGCUCACCAA GUCGAUGAUG   224
MycoSu13      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSui9      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu12      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu14      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSu15      1  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........     0
MycoSuis    187  UUAAAUUAAA GGAGGCUGCC GAAAGGUGG- CC-UCGCGGA UGAAUAGGAA UAUGUCCUAU UAGGUCGUUG GAGAGGUAAU GGCUCACCAA GCCGAUGAUG   284
MycoSui2    187  UUAAAUUAAA GGAGGAUGCC GCAAGGUGGC CU-UCGCGGG UGAAUAGGAA UAUGUCCUAU UAGAUAGUUG GAGAGGUAAU GGCUCACCAA GUCGAUGAUG   285
MycoSui3    187  TTAAATTAAA GGAGGATGCC GCAAGGTGG- CCTTCGCGGG TGAATAGGAA TATGTCCTAT TAGATAGTTG GAGAGGTAAT GGCTCACCAA GTCGATGATG   285
MycoSui4    187  TTAAATTAAA GGAGGGTGCC GCAAGGTGG- CCTTAGCGGC TCAATAGGTA TATGTCCTAT TAGATACTTG GAGAGGTAAC GGCTCACCAA GTCGATGATG   285
HmbFeli3    185  AGCUUUUAAA -GC-CU---- -UC-----G- G--GCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUAGUUG GCGGGAUAAA AGCCCACCAA GGCAAUGAUA   269
HmbCanis    171  AGCUUUUAAA -GC-CU---- -UC-----G- G--GCGCUGA GGGAUUGGGA UAUGCUCUAU UAGCUAGUUG GCGGGAUAAA AGCCCACCAA GGCAAUGAUA   255
MycCocco    186  GUUUUUUAAA -GG-AG---- -CA-----A- U--CCGCUUU GGGAUUGGAA UAUGCUCUAU UAGUUAGUUG GCGGGGUAAA GGCCCACCAA GACUAUGAUA   270
HmbMuris    170  UGGCUUUAAA -GG-CG---- -CA-----A- G--CCACUUG GAGAUUGGAG UAUUUUCUAU UAGCUAGUUG GCGGGAUAAU AGCCCACCAA GGCAGUGAUA   254
MycPneu5    190  AAAGUUGAAA -GGACCUG-- -CA---AGG- GU-UCGUUAU UUGAUGAGGG UGCGCCAUAU CAGCUAGUUG GUGGGGUAAC GGCCUACCAA GGCAAUGACG   280

                 301        311        321        331        341        351        361        371        381        391       400
                 |          |          |          |          |          |          |          |          |          |         |
MycoOvis    271  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   370
MycWenyo    243  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   342
MycoSu10      1  .......... .......... .......... .......... ...CTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    57
MycoSu21      1  .......... .......... .......... .......... ...CTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    57
MycoSu22      1  .......... .......... .......... .......... .......GCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    53
MycoSu16      1  .......... .......... .......... .......... .......... .......... .......... ......GGAA TTTTTTACA- TGGACGAAAG    23
MycoSu17      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACCA TGGACGGAAG    58
MycoSu11      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    58
MycoSu19      1  .......... .......... .......... .......... ...CTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    57
MycoSu20      1  .......... .......... .......... .......... ...CTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAA-G    56
MycoSu18      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAA-G    57
MycoSui6    290  GGTAGCTGGA CTGAGAGGTT GAACAGCCGC AATGGGATTG AGATATGGCC CATATTCCTA CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG   389
MycoSui7    290  GGTAGCTGGA CTGAGAGGTT GAACAGCCGC AATGGGATTG AGATATGGCC CATATTCCTA CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG   389
EperSui2    266  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   365
EperSuis    225  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   324
MycoSu13      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    58
MycoSui9      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    58
MycoSu12      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG    58
MycoSu14      1  .......... .......... .......... .......... ...CTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTTTCCA TGGACGAA-G    56
MycoSu15      1  .......... .......... .......... .......... ..CCTTGGCC CATATTCCTG CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAA-G    57
MycoSuis    285  GGUAGCUGGA CUGAGAGGUU GAACAGCCGC AAUGGGAUUG AGAAAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   384
MycoSui2    286  GGUAGCUGGA CUGAGCGGUU GAACAGCCGC AAUGGGAUUG AGAUAUGGCC CAUAUUCCUA CGGGAAGCAG CAGUGAGGAA UUUUUCACAA UGGACGAAAG   385
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Alignment 16S rRNA of *Mycoplasma suis*

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MycoSui3   286  GGTAGCTGGA CTGAGCGGTT GAACAGCCGC AATGGGATTG AGATATGGCC CATATTCCTA CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG   385
MycoSui4   286  GGTAGCTGGA CTGACCGGTT GAACAGCCGC AATGGGATTA AGATATGGCC CATATTCCTA CGGGAAGCAG CAGTGAGGAA TTTTTCACAA TGGACGAAAG   385
HmbFeli3   270  GAUAGCUGGU CUUAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   369
HmbCanis   256  GAUAGCUGGU CUUAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   355
MycCocco   271  GAUAGCUGGU CUUAGAGGAC GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGACGAAAG   370
HmbMuris   255  GAUAGCUGGU CUAAGAGGAU GAACAGCCAC AAUGGGAUUG AGAUACGGCC CAUAUUCCUA CGGGAAGCAG CAGUAGGGAA UCUUCCACAA UGGGCGAAAG   354
MycPneu5   281  UGUAGCUAUG CUGAGAAGUA GAAUAGCCAC AAUGGGACUG AGACACGGCC CAUACUCCUA CGGGAGGCAG CAGUAGGGAA UUUUUCACAA UGAGCGAAAG   380

                401         411         421         431         441         451         461         471         481         491       500
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis   371  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUCUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGCGC- --UAGGAAAU GA---GCGCG   461
MycWenyo   343  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUCUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGUGC- --UAGGAAAU GA---GCACG   433
MycoSu10    58  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   148
MycoSu21    58  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---TCGCG   148
MycoSu22    54  TCTGATGG-G AGCCTACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   144
MycoSu16    24  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   114
MycoSu17    59  TCTGATGGCA GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   150
MycoSu11    59  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   149
MycoSu19    58  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   148
MycoSu20    57  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   147
MycoSu18    58  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   148
MycoSui6   390  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   480
MycoSui7   390  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCTA- --CAGGAAAT GG---TCGCG   480
EperSui2   366  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUCUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGCGA- --CAGGAAAU GG---UCGCG   456
EperSuis   325  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUCUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGCGA- --CAGGAAAU GG---UCGCG   415
MycoSu13    59  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---CCGCG   149
MycoSui9    59  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---CCGCG   149
MycoSu12    59  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---CCGCG   149
MycoSu14    57  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---CCGCG   147
MycoSu15    58  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGG- --CAGGAAAT GG---CCGCG   148
MycoSuis   385  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUCUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGCGG- --CAGGAAAU GG---CCGCG   475
MycoSui2   386  UCUGAUGG-A GCAAUACCAC GUGAACGAUG AAGGUGUU-C UGAUUGUAAA GUUCUUUUAU UUAGGAAAAA -AAGCGCGA- --CAGGAAAU GG---UCGCG   476
MycoSui3   386  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTGTT-C TGATTGTAAA GTTCTTTTAT TTAGGAAAAA -AAGCGCGA- --CAGGAAAT GG---TCGCG   476
MycoSui4   386  TCTGATGG-A GCAATACCAC GTGAACGATG AAGGTCTT-C TGATTGTAAA GTTCATTTAT TTAGGAAAAA -AAGCGCGA- --CAGGAAAT GG---ACGCG   476
HmbFeli3   370  UCUGAUGG-A GCAAUACCAU GUGAACGAUG AAGGCCUUUU UGGUUGUAAA GUUCUUUUAC GAGGGAUA-- ---------- -----AUUAU GA---------   443
HmbCanis   356  UCUGAUGG-A GCAAUACCAU GUGAACGAUG AAGGCCUUUU UGGUUGUAAA GUUCUUUUAC GAGGGAUA-- ---------- -----AUUAU GA---------   429
MycCocco   371  UCUGAUGG-A GCAAUGCCAU GUGAACGAUG AAGGUCAUUU UGAUUGUAAA GUUCUUUUAG GAGGGAAA-- ---------- -----AUUAU GA---------   444
HmbMuris   355  CCUGAUGG-A GUGAUGCCAU GUGAACGAUG AAGGUCUUUU UGAUUGUAAA GUUCUUUUAU UGGGGAAA-- ---------- -----AUGAU GA---------   428
MycPneu5   381  CUUGAUGG-A GCAAUGCCGC GUGAACGAUG AAGGUCUUUA AGAUUGUAAA GUUCUUUUAU UUGGGAAGAA UGACUUUAGC AG---GU-AA --UGGCUAGA   473

                501         511         521         531         541         551         561         571         581         591       600
                |           |           |           |           |           |           |           |           |           |         |
MycoOvis   462  CCUUGAUGGU ACUA-AUUGA AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC AAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   560
MycWenyo   434  CCUUGAUGGU ACUA-AUUGA AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC GAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   532
MycoSu10   149  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   247
MycoSu21   149  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   247
MycoSu22   145  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   243
MycoSu16   115  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   213
MycoSu17   151  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   249
MycoSu11   150  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   248
MycoSu19   149  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   247
MycoSu20   148  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   246
MycoSu18   149  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   247
MycoSui6   481  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   579
MycoSui7   481  CCCTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   579
EperSui2   457  CCCUGAUUGU ACUA-AUUGA AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC GAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   555
EperSuis   416  CCCUGAUUGU ACUA-AUUGA AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC GAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   514
MycoSu13   150  CCTTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   248
MycoSui9   150  CCTTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   248
MycoSu12   150  CCTTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   248
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Alignment 16S rRNA of *Mycoplasma suis*

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MycoSu14   148  CCTTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   246
MycoSu15   149  CCTTGATTGT ACTA-ATTGA ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   247
MycoSuis   476  CCUUGAUUGU ACUA-AUUGA AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC GAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   574
MycoSui2   477  CCCUGAUUGU ACUA-AUUGC AUAAGUGACA GCUAACUAUG UGCCAGCAGC UGCGGUAAAA CAUAGGUCAC GAGCAUUAUC CGGAUUUAUU GGGCGUAAAG   575
MycoSui3   477  CCCTGATTGT ACTA-ATTGC ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   575
MycoSui4   477  CCCTGATTGT ACTA-ATTGG ATAAGTGACA GCTAACTATG TGCCAGCAGC TGCGGTAAAA CATAGGTCAC GAGCATTATC CGGATTTATT GGGCGTAAAG   575
HmbFeli3   444  ------UAGU ACUU-GCUGA AUAAGUGACA GCAAACUAUG UGCCAGCAGC UGCGGUAAUA CAUAGGUCGC GAGCAUUAUU CGGAUUUAUU GGGCGUAAAG   536
HmbCanis   430  ------UAGU ACUU-CGUGA AUAAGUGACA GCAAACUAUG UGCCAGCAGC UGCGGUAAUA CAUAGGUCGC AAGCAUUAUU CGGAUUUAUU GGGCGUAAAG   522
MycCocco   445  ------UGGU ACCU-CCUGA AUAAGUGACA GCAAACUAUG UGCCAGCAGC UGCGGUAAUA CAUAGGUCGC AAGCGUUAUU CGGAUUUAUU GGGCGUAAAG   537
HmbMuris   429  ------UGGU ACCC-AGUGA AUAAGUGACA GCAAACUAUG UGCCAGCAGC UGCGGUAAUA CAUAGGUCGC GAGCGUUAUU CGGAUUUAUU GGGCGUAAAG   521
MycPneu5   474  GUUUGACUGU ACCAUUUUGA AUAAGUGACG ACUAACUAUG UGCCAGCAGU CGCGGUAAUA CAUAGGUCGC AAGCGUUAUC CGGAUUUAUU GGGCGUAAAG   573

                      601        611        621        631        641        651        661        671        681        691       700
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   561  GAAGCGUAGG CGGGGA-GGC UGAUCCAUUG UUAAAGGCAU UUGCUCAACA AAUGUGUGCG AUGGAGAUCU CCUCCCUAGA GUUAAUCAGG GGGUACUGGA   659
MycWenyo   533  GAAGCGUAGG CGGGGA-GGU UGAUCCAUUG UUAAAAGCAU UUGCUUAACA AAUGUGUGCA UUGGAGAUCG CCUCCCUAGA GUUAAUCAGG GGGUACUGGA   631
MycoSu10   248  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   346
MycoSu21   248  GAAGCGTAGG CTGAAA-TGT GTATCCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   346
MycoSu22   244  GAAGCGTAAG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   342
MycoSu16   214  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   312
MycoSu17   250  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   348
MycoSu11   249  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   347
MycoSu19   248  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   346
MycoSu20   247  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   345
MycoSu18   248  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   346
MycoSui6   580  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   678
MycoSui7   580  GAAGCGTAGG CTGAAG-TGT GTATCCATTG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA CACTTCTAGA ATTAGTTAGA GGGCACTGGA   678
EperSui2   556  GAAGCGUAGG CUGAAG-UGU GUAUCCAUUG UUAAAAGUAC UUGCUUAACA AGUGUUCGCG GUGGAGAUUA CACUUCUAGA AUUAGUUAGA GGGCACUGGA   654
EperSuis   515  GAAGCGUAGG CUGAAG-UGU GUAUCCAUUG UUAAAAGUAC UUGCUUAACA AGUGUUCGCG GUGGAGAUUA CACUUCUAGA AUUAGUUAGA GGGCACUGGA   613
MycoSu13   249  GAAGCGTAGG CTGAAA-TGT GTATTCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGAAGATTA CATTTCTAGA ATTAGTTAGA GGGTACTGGA   347
MycoSui9   249  GAAGCGTAGG CTGAAA-TGT GTATTCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGAAGATTA CATTTCTAGA ATTAGTTAGA GGGTACTGGA   347
MycoSu12   249  GAAGCGTAGG CTGAAA-TGT GTATTCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGAAGATTA CATTTCTAGA ATTAGTTAGA GGGTACTGGA   347
MycoSu14   247  GAAGCGTAGG CTGAAA-TGT GTATTCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGAAGATTA CATTTCTAGA ATTAGTTAGA GGGTACTGGA   345
MycoSu15   248  GAAGCGTAGG CTGAAA-TGT GTATTCATTG TTAAAAATAT TTGCTTAACA AGTGTTCGCG GTGAAGATTA CATTTCTAGA ATTAGTTAGA GGGTACTGGA   346
MycoSuis   575  GAAGCGUAGG CUGAAA-UGU GUAUUCAUUG UUAAAAAUAU UUGCUUAACA AGUGUUCGCG GUGAAGAUUA CAUUUCUAGA AUUAGUUAGA GGGUACUGGA   673
MycoSui2   576  GGAGCGUAGG CUGAAG-UGU GUAUCCUUAG UUAAAAGUAC UUGCUUAACA AGUGUUCGCG GUGGAGAUUA CAGUUCUAGA AUUAGUUAGA GGGCACUGGA   674
MycoSui3   576  GGAGCGTAGG CTGAAG-TGT GTATCCTTAG TTAAAAGTAC TTGCTTAACA AGTGTTCGCG GTGGAGATTA ATCTTCTAGA ATTAGTTAGA GGGCACTGGA   674
MycoSui4   576  GCAGCGTAGG CAGAAG-TGT GTATCCATTG TTAAAACTAC TTGCTTAACA AGTGTTCGCG GTGCAGATTA CAATTCTAGA ATTAGTTAGA GGGGACTGGA   674
HmbFeli3   537  CAAGCGCAGG CGGAUGUUGU AAGUUCUGUG UUAAAUGCAG CUACUCAAUA GUUGUAUGCA CCGAAUACUA CAUGUCUAGA UUGUGGUAGG GAGUUUCGGA   636
HmbCanis   523  CAAGCGCAGG CGGAUG-UGU AAGUUCUGUG UUAAAUGCAG CUACUCAAUA GUUGUAUGCA CCGAAUACUA CAUGUCUAGA UUGUGGUAGG GAGUUUCGGA   621
MycCocco   538  CAAGCGCAGG CGGAUG-AAC AAGUUCUGUG UUAAAAGCAG CUGCUCAACA GUUGUUUGCA CCGAAUACUG UUCGUCUAGA AUGUGGUAGG AAGUUUUGGA   636
HmbMuris   522  CGAGCGCAGG CGGAUU-GGU AAGUUCUGUG UUAAAUGCAG CCGCUCAACG GUUGUAUGCG CAGAAUACUG CCUUUCUAGA AUACGGUAGG AAGUUUUGGA   620
MycPneu5   574  CAAGCGCAGG CGGAUU-GAA AAGUCUGGUG UUAAAGGCAG CUGCUUAACA GUUGUAUGCA UUGGAAACUA UUAAUCUAGA GUGUGGUAGG GAGUUUUGGA   672

                      701        711        721        731        741        751        761        771        781        791       800
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   660  AUUCAAUGUG UAGCGGUGGA AUGCGUAGAU AUAUUGAGGA ACACCAGAGG CUAAGGCGAG UACCUGGGAU AUA-ACUGAC GCUGAGGCUU GAAAGCGUGG   758
MycWenyo   632  AUUCAAUGUG UAGCGGUGGA AUGCGUAGAU AUAUUGAGGA ACACCAGAGG CUAAGGCGAG UGCCUAGGAU AUA-ACUGAC GCUGAGGCUU GAAAGCGUGG   730
MycoSu10   347  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   445
MycoSu21   347  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   445
MycoSu22   343  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   441
MycoSu16   313  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   411
MycoSu17   349  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   447
MycoSu11   348  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   446
MycoSu19   347  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   445
MycoSu20   346  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   444
MycoSu18   347  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   445
MycoSui6   679  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG CGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   777
MycoSui7   679  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCGGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   777
EperSui2   655  AUUCAAUGUG UAGUGGUGGA AUACGUAGAU AUAUUGAGGA ACACCAGAGG CUAAGGCGAG UGCCUGGGAC AUA-AUUGAC GCUGAGGCUU GAAAGCGUGG   753
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Alignment 16S rRNA of *Mycoplasma suis*

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EperSuis   614  AUUCAAUGUG UAGUGGUGGA AUACGUAGAU AUAUUGAGGA ACACCAGAGG CUAAGGCGAG UGCCUGGGAC AUA-AUUGAC GCUGAGGCUU GAAAGCGUGG   712
MycoSu13   348  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGAAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   446
MycoSui9   348  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGAAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   446
MycoSu12   348  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGAAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   446
MycoSu14   346  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGAAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   444
MycoSu15   347  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGAAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   445
MycoSuis   674  AUUCAAUGUG UAGUGGUGGA AUACGUAGAU AUAUUGAGGA ACACCAGAGG CUAAGGCGAG UGCCUGGAAC AUA-AUUGAC GCUGAGGCUU GAAAGCGUGG   772
MycoSui2   675  AUUCAAUGUG UAGUGGUGGA AUACGUAGAU AUAUUGAGGA ACACCAGAGG CUAGGGCGAG UGCCUGGGAC AUA-AUUGAC GCUGAGGCUU GAAAGCGUGG   773
MycoSui3   675  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAGGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   773
MycoSui4   675  ATTCAATGTG TAGTGGTGGA ATACGTAGAT ATATTGAGGA ACACCAGAGG CTAAGGCGAG TGCCTGGGAC ATA-ATTGAC GCTGAGGCTT GAAAGCGTGG   773
HmbFeli3   637  AUUAAGCAUG GAGCGGUGGA AUGUGUAGAU AUGCUUAAGA ACACCAGAGG CGAAGGCGGA AACUUAGGCC -AUAAAUGAC GUUUAGGCUU GAAAGUGUGG   735
HmbCanis   622  AUUAAGCAUG GAGCGGUGGA AUGUGUAGAU AUGCUUAAGA ACACCAGAGG CGAAGGCGGA AACUUAGGCC -AUAAAUGAC GCUUAGGCUU GAAAGUGUGG   720
MycCocco   637  AUUAAAUAUG GAGCGGUGGA AUGUGUAGAU AUAUUUAAGA ACACCAGAGG CGAAGGCGAA AACUUAGGCC -AUUAUUGAC GCUUAGGCUU GAAAGUGUGG   735
HmbMuris   621  AUUGAAUGUG GAGCGGUGGA AUGUGUAGAU AUAUUCAAGA ACACCAGAGG CGAAGGCGAA AACUUAGGCC -GAUAUUGAC GCUUAGGCUC GAAAGUGUGG   719
MycPneu5   673  AUUUCAUGUG GAGCGGUGAA AUGCGUAGAU AUAUGAAGGA ACACCAGUGG CGAAGGCGAA AACUUAGGCC -AUUACUGAC GCUUAGGCUU GAAAGUGUGG   771

                    801         811         821         831         841         851         861         871         881         891       900
                    |           |           |           |           |           |           |           |           |           |         |
MycoOvis   759  GGAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGCCGUA AACGAUGAGC AUUAGGUAUU UGAU-GUUAG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   856
MycWenyo   731  GGAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGCCGUA AACGAUGAGC AUUGGGCAUU UGAC-AUCUG -GUCGAGUGC UGUAGCUAAC GCGUUAAAUG   828
MycoSu10   446  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   543
MycoSu21   446  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   543
MycoSu22   442  GTAGCAAATG GGATTAGATA CCACAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   539
MycoSu16   412  GTAGCAAATG GGATTAGATA CCCCASTAGT CCACGCCGTA AACSATGGGT ATTASTCATT TGAA-TTTAA -STTGAGTGA TGTAGCTAAC GCSTTTAATA   509
MycoSu17   448  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   545
MycoSu11   447  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   544
MycoSu19   446  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   543
MycoSu20   445  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   542
MycoSu18   446  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   543
MycoSui6   778  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   875
MycoSui7   778  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAA-TTTAA -GTTGAGTGA TGTAGCTAAC GCGTTAAATA   875
EperSui2   754  GUAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGCCGUA AACGAUGGGU AUUAGUCAUU UGAA-UUUAA -GUUGAGUGA UGUAGCUAAC GCGUUAAAUA   851
EperSuis   713  GUAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGCCGUA AACGAUGGGU AUUAGUCAUU UGAA-UUUAA -GUUGAGUGA UGUAGCUAAC GCGUUAAAUA   810
MycoSu13   447  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGGA-TTAAG -ACTGAGTGA TGTAGCTAAC GCGTTAAATA   544
MycoSui9   447  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGGATTTAA- GACTGAGTGA TGTAGCTAAC GCGTTAAATA   545
MycoSu12   447  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGGATTTAA- GACTGAGTGA TGTAGCTAAC GCGTTAAATA   545
MycoSu14   445  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGGATTTAA- GACTGAGTGA TGTAGCTAAC GCGTTAAATA   543
MycoSu15   446  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACCCCGTA AACGATGGGT ATTAGTCATT TGGATTTAA- GACTGAGTGA TGTAGCTAAC GCGTTAAATA   544
MycoSuis   773  GUAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGCCGUA AACGAUGGGU AUUAGUCAUU UGGAUUUAA- GACUGAGUGA UGUAGCUAAC GCGUUAAAUA   871
MycoSui2   774  GUAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACGUCGUA AUCGAUGGGU AUUAGUCAUU UGAAU-UUA- AGUUGAGUGA UGUAGCUAAC GCGUUAAAUA   871
MycoSui3   774  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGTCGTA ATCGATGGGT ATTAGTCATT TGAAT-TTA- AGTTGAGTGA TGTAGCTAAC GCGTTAAATA   871
MycoSui4   774  GTAGCAAATG GGATTAGATA CCCCAGTAGT CCACGCCGTA AACGATGGGT ATTAGTCATT TGAAT-TTA- AGATGAGTGA TGTAGCTAAC GCGTTAAATA   871
HmbFeli3   736  GGAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGCUUUA- GCUUUAGUGU UGUAGCUUAC GCGUUAAAUA   834
HmbCanis   721  GGAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACACCGUA AACGAUGGGU AUUAGAUAUU AGGGCUUUA- GCUUUAGUGU UGUAGCUUAC GCGUUAAAUA   819
MycCocco   736  GUAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACACCGUA AACGAUGGGU AUUAGGUGCC GGGGUUAGA- GCUUCGGUGC UGUAGCUUAC GUGUUAAAUA   834
HmbMuris   720  GGAGCAAAUG GGAUUAGAUA CCCCAGUAGU CCACACCGUA AACGAUGGAU AUUAGAUGUU GGGACUUGA- GUCUCAGCGU UGUAGCUUAC GUGUUAAAUA   818
MycPneu5   772  GGAGCAAAUA GGAUUAGAUA CCCUAGUAGU CCACACCGUA AACGAUAGAU ACUAGCUGUC GGGGCGAUC- CCCUCGGUAG UGAAGUUAAC ACAUUAAGUA   870

                    901         911         921         931         941         951         961         971         981         991      1000
                    |           |           |           |           |           |           |           |           |           |         |
MycoOvis   857  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   955
MycWenyo   829  CUCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   927
MycoSu10   544  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   642
MycoSu21   544  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   642
MycoSu22   540  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   638
MycoSu16   510  CCCCGCCYGG GTAGYATATA TGCAAATATG AAACYCAA-- GAAATTGACG GGGACGTGCA CAAGTGRTGG ACCATTGTGC CTAATTATAT AATACTCGCC   607
MycoSu17   546  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGGTGC TTAATTCGAT AATACACGCA   644
MycoSu11   545  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   643
MycoSu19   544  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   642
MycoSu20   543  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   641
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Alignment 16S rRNA of *Mycoplasma suis*

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MycoSu18   544  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   642
MycoSui6   876  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT TCTACACGCA   974
MycoSui7   876  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT TCTACACGCA   974
EperSui2   852  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU CAUACACGCA   950
EperSuis   811  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU CAUACACGCA   909
MycoSu13   545  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAATTTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   643
MycoSui9   546  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   644
MycoSu12   546  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   644
MycoSu14   544  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   642
MycoSu15   545  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT AATACACGCA   643
MycoSuis   872  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGCA   970
MycoSui2   872  CCCCGCCUGG GUAGUAUAUA UGCAAAUAUG AAACUCAAA- GAAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU CAUACACGCA   970
MycoSui3   872  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGTTGC TTAATTCGAT CATACACGCA   970
MycoSui4   872  CCCCGCCTGG GTAGTATATA TGCAAATATG AAACTCAAA- GAAATTGACG GGGACCTGAA CAAGTGGTGG AGCATGATGC TTAATTCGAT CATACACGCT   970
HmbFeli3   835  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   933
HmbCanis   820  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAU AAUACACGAA   918
MycCocco   835  CCCCGCCUGG GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AACAUGUUGC UUAAUUCGAU AAUACACGAA   933
HmbMuris   819  UCCCGCCUGA GUAGUACAUA UGCAAAUAUG AAACUCAAA- GGAAUUGACG GGGACCUGAA CAAGUGGUGG AACAUGUUGC UUAAUUCGAC AAUACACGAA   917
MycPneu5   871  UCUCGCCUGG GUAGUACAUU CGCAAGAAUG AAACUCAAAC GGAAUUGACG GGGACCCGCA CAAGUGGUGG AGCAUGUUGC UUAAUUCGAC GGUACACGAA   970

                 1001       1011       1021       1031       1041       1051       1061       1071       1081       1091      1100
                 |          |          |          |          |          |          |          |          |          |         |
MycoOvis   956  AAACCUUACC AAGGCUUGUU AUCUACUGCA AA-ACUAUAG AAAUAUGGUG G-AGUAU--A UCAGUAAGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1051
MycWenyo   928  AAACCUUACC AAGGCUUGUA AUCUAUUGCG AA-GCUAUAG AAAUAUAGUG G-AGGUU--A UCAGUAAGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1023
MycoSu10   643  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   738
MycoSu21   643  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   738
MycoSu22   639  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   734
MycoSu16   608  AAACCTTACC AAGGTTAGTA ATCTGCTGCC AA-GCTAAAT CAATAAATTG A-ATGTC--A TCCGAATAAC CGGTGGTGCA TGGCTGTCGT CAACTCGTGT   703
MycoSu17   645  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAACTCGTGT   740
MycoSu11   644  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAACTCGTGT   739
MycoSu19   643  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   738
MycoSu20   642  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   737
MycoSu18   643  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   738
MycoSu6    975  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TCAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT  1070
MycoSui7   975  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AA-GCTATAG AAATATAGTG G-AGGCT--A TTAGAATGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT  1070
EperSui2   951  AAACCUUACC AAGGCUUGCA AUCUUCUGCA AA-GCUAUAG AAAUAUAGUG G-AGGCU--A UCAGAAUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1046
EperSuis   910  AAACCUUACC AAGGCUUGCA AUCUUCUGCA AA-GCUAUAG AAAUAUAGUG G-AGGCU--A UCAGAAUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1005
MycoSu13   644  AAACCTTACC GAGGCTTGCA ATCCTCCGCA AC-GCTATAT AAATATAGTT G-AGGTT--A TCGGAGTGAC AGGGGGTGCA TGGCTGTCGT CAGCTCGTGT   739
MycoSui9   645  AAACCTTACC GAGGCTTGCA ATCCTCCGCA AC-GCTATAT AAATATAGTT G-AGGTT--A TCGGAGTGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   740
MycoSu12   645  AAACCTTACC GAGGCTTGCA ATCCTCCGCA AC-GCTATAT AAATATAGTT G-AGGTT--A TCGGAGTGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   740
MycoSu14   643  AAACCTTACC GAGGCTTGCA ATCCTCCGCA AC-GCTATAT AAATATAGTT G-AGGTT--A TCGGAGTGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   738
MycoSu15   644  AAACCTTACC GAGGCTTGCA ATCCTCCGCA AC-GCTATAT AAATATAGTT G-AGGTT--A TCGGAGTGAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT   739
MycoSuis   971  AAACCUUACC GAGGCUUGCA AUCCUCCGCA AC-GCUAUAU AAGUAUAGUU G-AGGUU--A UCGGAGUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1066
MycoSui2   971  AAACCUUACC AAGGCUUGCA AUCUUCUGCA ACAG.UAGAU AAAUAUAGUG G-AGGAU--A UCAGAGUCAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1066
MycoSui3   971  AAACCTTACC AAGGCTTGCA ATCTTCTGCA AC-CGTAGAT AAGTATAGTG G-AGGAT--A TCCGAGTCAC AGGTGGTGCA TGGCTGTCGT CAGCTCGTGT  1066
MycoSui4   971  AAACCTTACC AAGGCTTGCA ATCATCTGCA AC-GCTCCAT AACTATAGTG G-AGGCA--A TCACACTGAC AGGTGGAGCA TGGCTGTCGT CAGCTCGTGT  1066
HmbFeli3   934  AAACCUUACC AAGGUUUGAC AUCCCUCGCA AA-GCUAUAG AAAUAUAGUA GA-G-GUUA- UCGAGGUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1029
HmbCanis   919  AAACCUUACC AAGGUUUGAC AUCCCUCGCA AA-GCUAUAG AAAUAUAGUA GA-G-GUUA- UCGAGGUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1014
MycCocco   934  GAACCUUACC AAGGUUUGAC AUCCCUCGCA AA-ACCAUAG AAAUAUGGCG G-AG-GUUA- UCGAGGUGAC AGGUGGUGCA UGGUUGUCGU CAGCUCGUGU  1029
HmbMuris   918  AAACCUUACC AAGAUUUGAC AUCCCCUGCG AA-GCUUUAG AAAUAAAGUG G-AG-GUUA- UCAGGGUGAC AGGUGGUGCA UGGCUGUCGU CAGCUCGUGU  1013
MycPneu5   971  AAACCUUACC UAGACUUGAC AUCCUUGGCA AA-GUUAUGG AAACAUAAUG G-AG-GUUA- ACCGAGUGAC AGGUGGUGCA UGGUUGUCGU CAGCUCGUGU  1066

                 1101       1111       1121       1131       1141       1151       1161       1171       1181       1191      1200
                 |          |          |          |          |          |          |          |          |          |         |
MycoOvis  1052  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU ACUCUCUAG- UU-AAUU-AG UUCUAGAGUG ACUGA-AUCG UAAGAU-AUA GGAAGGAUGG  1146
MycWenyo  1024  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU ACUCUCUAG- UU-ACUU-AG UUCUAGAGUG ACUGA-AUCG UAAGAU-AUA GGAAGGAUGG  1118
MycoSu10   739  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATAAG ACTGA-GTCG TAAGAT-CTA GGAAGGATGG   833
MycoSu21   739  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATAAG ACTGA-GTCG TAAGAT-CTA GGAAGGATGG   833
MycoSu22   735  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATAAG ACTGA-GTCG TAAGAT-CTA GGAAGGATGG   829
MycoSu16   704  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   798
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Alignment 16S rRNA of *Mycoplasma suis*

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MycoSu17   741  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   835
MycoSu11   740  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   834
MycoSu19   739  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTATAT-G ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   832
MycoSu20   738  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTATAT-G ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   831
MycoSu18   739  CTTGAGATGT TTGGTTAAGT CCCGT-ACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   832
MycoSui6  1071  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG  1165
MycoSui7  1071  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG  1165
EperSui2  1047  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU UCAUAUUAGU UG-UUU--AG UUCUAAUAUG ACUGA-AUCG UAAGAU-CUA GGAAGGAUGG  1141
EperSuis  1006  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU UCAUAUUAGU UG-UUU--AG UUCUAAUAUG ACUGA-AUCG UAAGAU-CUA GGAAGGAUGG  1100
MycoSu13   740  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCTTATTAGT TG-CTT--AG TTCTAATAAG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   834
MycoSui9   741  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCTTATTAGT TG-CTT--AG TTCTAATAAG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   835
MycoSu12   741  CTTGAGATGT TTGATTAT-T CCCGTAACGA GCGCAACCCT TCTTATTAGT TG-CTT--AG TTCTAATAAG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   834
MycoSu14   739  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCTTATTAGT TG-CTT--AG TTCTAATAAG ACTGA-ATCG TAAGAT-CTA G-AAGGATGG   832
MycoSu15   740  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCTTATTAGT TG-CTT--AG TTCTAATAAG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG   834
MycoSuis  1067  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU UCUUAUUAGU UG-CUU--AG UUCUAAUAAG ACUGA-AUCG UAAGAU-CUA GGAAGGAUGG  1161
MycoSui2  1067  CUUGAGAUGU UUGGUUAAGU CCCGUAACGA GCGCAACCCU UCAUAUUAGU UG-UUU--AG UUCUAAUAUG ACUGA-AUCG UAAGAU-CUA GGAAGGAUGG  1161
MycoSui3  1067  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGT TG-TTT--AG TTCTAATATG ACTGA-ATCG TAAGAT-CTA GGAAGGATGG  1161
MycoSui4  1067  CTTGAGATGT TTGGTTAAGT CCCGTAACGA GCGCAACCCT TCATATTAGA TG-TTT--AG TTCTAATATG ACAGA-ATCG TAAGAT-CTA GGACGGATGG  1161
HmbFeli3  1030  CUUGAGAUGU UUGGUUAAGU CCCGCAACGA GCGCAACCCC ACUCUUUAG- UUA--C-UUG -UCUAAAGAG ACUGC-ACAG UAAUGU-AGA GGAAGGAUGG  1122
HmbCanis  1015  CUUGAGAUGU UUGGUUAAGU CCCGCAACGA GCGCAACCCC ACUCUUUAG- UUA--C-UUG -UCUAAAGAG ACUGC-ACAG UAAUGU-AGA GGAAGGAUGG  1107
MycCocco  1030  CAUGAGAUGU UUGGUUAAGU CCCGCAACGA GCGCAACCCU ACUCUUUAG- UUA--CUUUA -UCUAAAGAG ACUGA-ACAG UAAUGU-AUA GGAAGGAUGG  1123
HmbMuris  1014  CAUGAGAUGU CUGGUUAAGU CCUGAAACGA GCGCAACCCU ACUCUUUAG- UUA--A-CUU -UCUAAAGAG ACUGA-ACAG UAAUGU-AUA GGAAGGAUGG  1106
MycPneu5  1067  CGUGAGAUGU UGGGUUAAGU CCCGCAACGA GCGCAACCCU UAUCGUUAG- UUA--CAUUG -UCUAGCGAG ACUGCUAAUG CAAAUUG-GA GGAAGGAAGG  1161

                1201       1211       1221       1231       1241       1251       1261       1271       1281       1291      1300
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis  1147  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGUAGG UACAAUGUGU UGCAAUCUAG -CGAUAGUGA GCUAAUCACC  1245
MycWenyo  1119  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGUAGG UACAAUGUGU UGCAAACUAG -CGAUAGUGA GCCAAUCACC  1217
MycoSu10   834  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   932
MycoSu21   834  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   932
MycoSu22   830  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   928
MycoSu16   799  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   897
MycoSu17   836  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   934
MycoSu11   835  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   933
MycoSu19   833  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   931
MycoSu20   832  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   930
MycoSu18   833  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC   931
MycoSui6  1166  GGCCAAGTTA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC  1264
MycoSui7  1166  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT TACAATCTAG -CGATAGTGA GTTAATCACC  1264
EperSui2  1142  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGUAGA UACAAUGUGU UACAAUCUAG -CGAUAGUGA GUUAAUCACC  1240
EperSuis  1101  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGUAGA UACAAUGUGU UACAAUCUAG GCGAUAGUGA GUUAAUCACC  1200
MycoSu13   835  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTCGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT GACAATCTAG -CGATAGTGA GTCAATCACC   933
MycoSui9   836  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTCGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT GACAATCTAG -CGATAGTGA GTCAATCACC   934
MycoSu12   835  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTCGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT GACAATCTAG -CGATAGTGA GTCAATCACC   933
MycoSu14   833  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTCGGGCTGC AAACGTGCTA CAATGGTAGA TACAATGTGT GACAATCTAG -CGATAGTGA GTCAATCACC   931
MycoSu15   835  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTCGGGCTGC AACCGTGCTA CAATGGTAGA TACAATGTGT GACAATCTAG -CGATAGTGA GTCAATCACC   933
MycoSuis  1162  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUCGGGCUGC GAACGUGCUA CAAUGGUAGA UACAAUGUGU GACAAUCUAG -CGAUAGUGA GUCAAUCACC  1260
MycoSui2  1162  GGCCAAGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUU CAAUGGUAGA UUCAAUGUGU GACAAUCUAG -CGAUAGUGA GUUAAUCACC  1260
MycoSui3  1162  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTT CAATGGTAGA TTCAATGTGT GACAATCTAG -CGATAGTGA GTTAATCACC  1260
MycoSui4  1162  GGCCAAGTCA AGTCATCATG CCCCTTATGC CTTGGGCTGC AAACGTGCTT CAATGGTAGA TACAATGTGT CACAATCTAG -CGATAGTGA GTTAATCACC  1260
HmbFeli3  1123  GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCGAA CACAAUGUGU UGCAAACCAG -CGAUGGUAA GCUAAUCACC  1221
HmbCanis  1108  GAUCACGUCA AGUCAUCAUG CCCCUUAUGC CUUGGGCUGC AAACGUGCUA CAAUGGCGAA CACAAUGUGU UGCAAACCAG -CGAUGGUAA GCUAAUCACC  1206
MycCocco  1124  GAUCACGUCA AAUCAUCAUG CCCCUUAUGC CUUGGGCCGC AAACGUGUUA CAAUGGGAGG UACAAUGUGU CGCAAACUAG -CGAUAGUAA GCUAAUCAC-  1221
HmbMuris  1107  GAUCACGUCA AGUCAUCAUG CCCCUUAUAU CUUGGGCCGC AAACGUGUUA CAAUGGUGGG UACAACGUGU CGCAAGCCAG -CGAUGGCAA GCCAAUCAC-  1204
MycPneu5  1162  GAUGACGUCA AAUCAUCAUG CCCCUUAUGU CUAGGGCUGC AAACGUGCUA CAAUGGCCAA UACAAACAGU CGCCAGCUUG -UAAAAGUGA GCAAAUCUG-  1259

                1301       1311       1321       1331       1341       1351       1361       1371       1381       1391      1400
                |          |          |          |          |          |          |          |          |          |         |
MycoOvis  1246  GAAA-ACCUA UCUCAGUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG UUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1344
MycWenyo  1218  UAAA-GCCUA UCUCAGUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG UUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1316
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Alignment 16S rRNA of *Mycoplasma suis*

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MycoSu10    933  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1007
MycoSu21    933  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1007
MycoSu22    929  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1003
MycoSu16    898  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........   972
MycoSu17    935  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1009
MycoSu11    934  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1008
MycoSu19    932  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCCATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1006
MycoSu20    931  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1005
MycoSu18    932  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1006
MycoSui6   1265  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCT GTGTCAGCTA TATCAGGGTG AATACGTTCC  1363
MycoSui7   1265  TAAA-GTCCA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCT GTGTCAGCTA TATCAGGGTG AATACGTTCC  1363
EperSui2   1241  UAAA-GUCUA UCUCAGUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG AUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1339
EperSuis   1201  UAAA-GUCUA UCUCAGUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG AUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1299
MycoSu13    934  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1008
MycoSui9    935  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1009
MycoSu12    934  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1008
MycoSu14    932  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC GTAAGG.... .......... ..........  1006
MycoSu15    934  TAAA-GTCTA TCTCAGTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCC .......... .......... ..........  1002
MycoSuis   1261  UAAA-GUCUA UCUCAGUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG AUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1359
MycoSui2   1261  UAAA-GUCUA UCUCACUCCG GAUAAAAGGC UGCAAUUCGC CUAUUUGAAG AUGGAAUCAC UAGUAAUCCU GUGUCAGCUA UAUCAGGGUG AAUACGUUCC  1359
MycoSui3   1261  TAAA-GTCTA TCTCACTCCG GATAAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCT GTGTCAGCTA TATCAGGGTG AATACGTTCC  1359
MycoSui4   1261  TAAA-GTCTA TCTCACTCCG GATTAAAGGC TGCAATTCGC CTATTTGAAG ATGGAATCAC TAGTAATCCT GTGTCAGCTA TATCAGGGTG AATACGTTCC  1359
HmbFeli3   1222  -AAA-UUUCG UCUCAGUUCG GAUAGGAGGC UGCAAUUCGC CUCCUUGAAG UUGGAAUCAC UAGUAAUCCC GUGUCAGCUA UAUCGGGGUG AAUCCGUUCC  1319
HmbCanis   1207  -AAA-UUUCG UCUCAGUUCG GAUAGGAGGC UGCAAUUCGC CUCCUUGAAG UUGGAAUCAC UAGUAAUCCC GUGUCAGCUA UAUCGGGGUG AAUCCGUUCC  1304
MycCocco   1222  UAAA-GCCUC UCCCAGUUCG GAUAAAAGGC UGCAAUUCGC CUUUUUGAAG UUGGAAUCAC UAGUAAUCCC GUGUCAGCUA UAUCGGGGUG AAUACGUUCC  1320
HmbMuris   1205  UAAAAGCCCA UCCCAGUCCG GAUAAAAGGC UGCAAUUCGC CUUUUUGAAG UUGGAAUCAC UAGUAAUCCC GUGUCAGCCA UAUCGGGGUG AAUACGUUCC  1304
MycPneu5   1260  UAAA-GUUGG UCUCAGUUCG GAUUGAGGGC UGCAAUUCGU CCUCAUGAAG UCGGAAUCAC UAGUAAUCGC GAAUCAGCUA UGUCGCGGUG AAUACGUUCU  1358

                      1401       1411       1421       1431       1441       1451       1461       1471       1481       1491      1500
                      |          |          |          |          |          |          |          |          |          |         |
MycoOvis   1345  CAGGUCUUGU ACACACCGCC CGUCAAACUA UGAAAGAAAG UAUCAGUCAA AACCGCA-U- ------UCAA ------UUGU GUCUAGAUUG GUAAUUUUGA  1430
MycWenyo   1317  CAGGUCUUGU ACACACCGCC CGUCAAACUA CGAAAGAAAG UACUAGUCAA AACCACA-U- ------UUAA ------UUGU GUCUAGAUUG GUAAUUU...  1399
MycoSu10   1008  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1007
MycoSu21   1008  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1007
MycoSu22   1004  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1003
MycoSu16    973  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........   972
MycoSu17   1010  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1009
MycoSu11   1009  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1008
MycoSu19   1007  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1006
MycoSu20   1006  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1005
MycoSu18   1007  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1006
MycoSui6   1364  CAGGTCTTGT ACACACCGCC CGTCAAACTA CGAAAGAAAG TACTAATTAA AACCGTA-T- ------TTAA ------TTAC GTCTAGATTG GTAATTTTGA  1449
MycoSui7   1364  CAGGTCTTGT ACACACCGCC CGTCAAACTA CGAAAGAAAG TACTAATTAA AACCGTA-T- ------TTAA ------TTAC GTCTAGATTG GTAATTTTGA  1449
EperSui2   1340  CAGGUCUUGU ACACACCGCC CGUCAAACUA CGAAAGAAAG UACUAAUUAA AACCGUA-U- ------UUAA ------UUAC GUCUAGAUUG GUAAUUUUGA  1425
EperSuis   1300  CAGGUGUUGU ACACACCGCC CGUCAAACUA CGAAAGAAAG UACUAAUUAA AACCGUA-U- ------UUAA ------UUAC GUCUAGAUU. ..........  1374
MycoSu13   1009  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1008
MycoSui9   1010  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1009
MycoSu12   1009  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1008
MycoSu14   1007  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1006
MycoSu15   1003  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........  1002
MycoSuis   1360  CAGGUCUUGU ACACACCGCC CGUCAAACUA CGAAAGAAAG UACUAAUUAA AACCGUA-U- ------UUAA ------UUAC GUCUAGAUUG GUAAUUUUGA  1445
MycoSui2   1360  CAGGUCUUGU ACACACCGCC CGUCAAACUA CGAAAGAAAG UACUAAUUAA AACCGUA-U- ------UUAC GUCUAGAUUG GUAAUUUUGA  1445
MycoSui3   1360  CAGGTCTTGT ACACACCGCC CGTCAAACTA CGAAAGAAAG TACTAATTAA AACCGTA-T- ------TTAA ------TTAC GTCTAGATTG GTAATTTTGA  1445
MycoSui4   1360  CAGGTCTTGT ACACACCGCC CGTCAAACTA CGAAAGAAAG TACTAATTTA AACCGTA-T- ------TTAA ------TTAC GTCTAGATTG GTAATTTTGA  1445
HmbFeli3   1320  CAGGUCUUGU ACACACCGCC CGUCAAACUA UGAGAGGAGU GGGCAUUUAA AAAUACA-U. .......... .......... .......... ..........  1377
HmbCanis   1305  CAGGUCUUGU ACACACCGCC CGUCAAACUA UGAGAGGAGU GGGCAUUUAA AAAUACA-U- ------UUAU ------UUGU AUCUAGAGUG AACAU-CUGA  1389
MycCocco   1321  CAGGUCUUGU ACACACCGCC CGUCAAACUA UGAGAG.... .......... .......... .......... .......... .......... ..........  1356
HmbMuris   1305  CAGGUCUUGU ACACACCGCC CGUCAAACUA UGAGAGGGAG AGGCAUUCGA AAACGCA-U- ------UCAU ------UUGC GUCUAGAAUG AAUUUUCCGA  1390
MycPneu5   1359  CGGGUCUUGU ACACACCGCC CGUCAAACUA UGAAAGCUGG UAAUAUUUAA AAACGUGUUG CUAACCAUUA GGAAGCGCAU GUCAAGGAUA GCACCGGUGA  1458
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Alignment 16S rRNA of *Mycoplasma suis*

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                1501       1511       1521       1531       1541       1551
                |          |          |          |          |          |
MycoOvis  1431  UUGGAGUUAA GUCGUAACAA GGUAACCG.. .......... .......... ....  1458
MycWenyo  1400  .......... .......... .......... .......... .......... ....  1399
MycoSu10  1008  .......... .......... .......... .......... .......... ....  1007
MycoSu21  1008  .......... .......... .......... .......... .......... ....  1007
MycoSu22  1004  .......... .......... .......... .......... .......... ....  1003
MycoSu16   973  .......... .......... .......... .......... .......... ....   972
MycoSu17  1010  .......... .......... .......... .......... .......... ....  1009
MycoSu11  1009  .......... .......... .......... .......... .......... ....  1008
MycoSu19  1007  .......... .......... .......... .......... .......... ....  1006
MycoSu20  1006  .......... .......... .......... .......... .......... ....  1005
MycoSu18  1007  .......... .......... .......... .......... .......... ....  1006
MycoSui6  1450  TTGGAGTTAA GTCGTAACGT AACCGTAAAG .......... .......... ....  1479
MycoSui7  1450  TTGGAGTTAA GTCGTAACGT AACCGTAAAG .......... .......... ....  1479
EperSui2  1426  UUGGAGUUAA G......... .......... .......... .......... ....  1436
EperSuis  1375  .......... .......... .......... .......... .......... ....  1374
MycoSu13  1009  .......... .......... .......... .......... .......... ....  1008
MycoSui9  1010  .......... .......... .......... .......... .......... ....  1009
MycoSu12  1009  .......... .......... .......... .......... .......... ....  1008
MycoSu14  1007  .......... .......... .......... .......... .......... ....  1006
MycoSu15  1003  .......... .......... .......... .......... .......... ....  1002
MycoSuis  1446  UUGGAGUUAA GUCGUAACAA GGU....... .......... .......... ....  1468
MycoSui2  1446  UUGGAGUUAA GUCGUAACAA GGUAGCCG.. .......... .......... ....  1473
MycoSui3  1446  TTGGAGTTAA GTCGTAACAA GGTAGCCG.. .......... .......... ....  1473
MycoSui4  1446  TTGGAGTTAA GTCGTAACAA GGTAGCCG.. .......... .......... ....  1473
HmbFeli3  1378  .......... .......... .......... .......... .......... ....  1377
HmbCanis  1390  UUGGAGUU.. .......... .......... .......... .......... ....  1397
MycCocco  1357  .......... .......... .......... .......... .......... ....  1356
HmbMuris  1391  UUGGAGUUAA G......... .......... .......... .......... ....  1401
MycPneu5  1459  UUGGAGUUAA GUCGUAACAA GGUACCCCUA CGAGAACGUG GGGGUGGAUC ACCU  1512
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- Assistant, Sondheim, Germany
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- Chemie Brunschwig AG, Basel, Switzerland
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- Vadaux Eppendorf, Schönenbuch, Basel, Switzerland
- Zeiss, Carl Zeiss AG, Feldbach, Switzerland

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